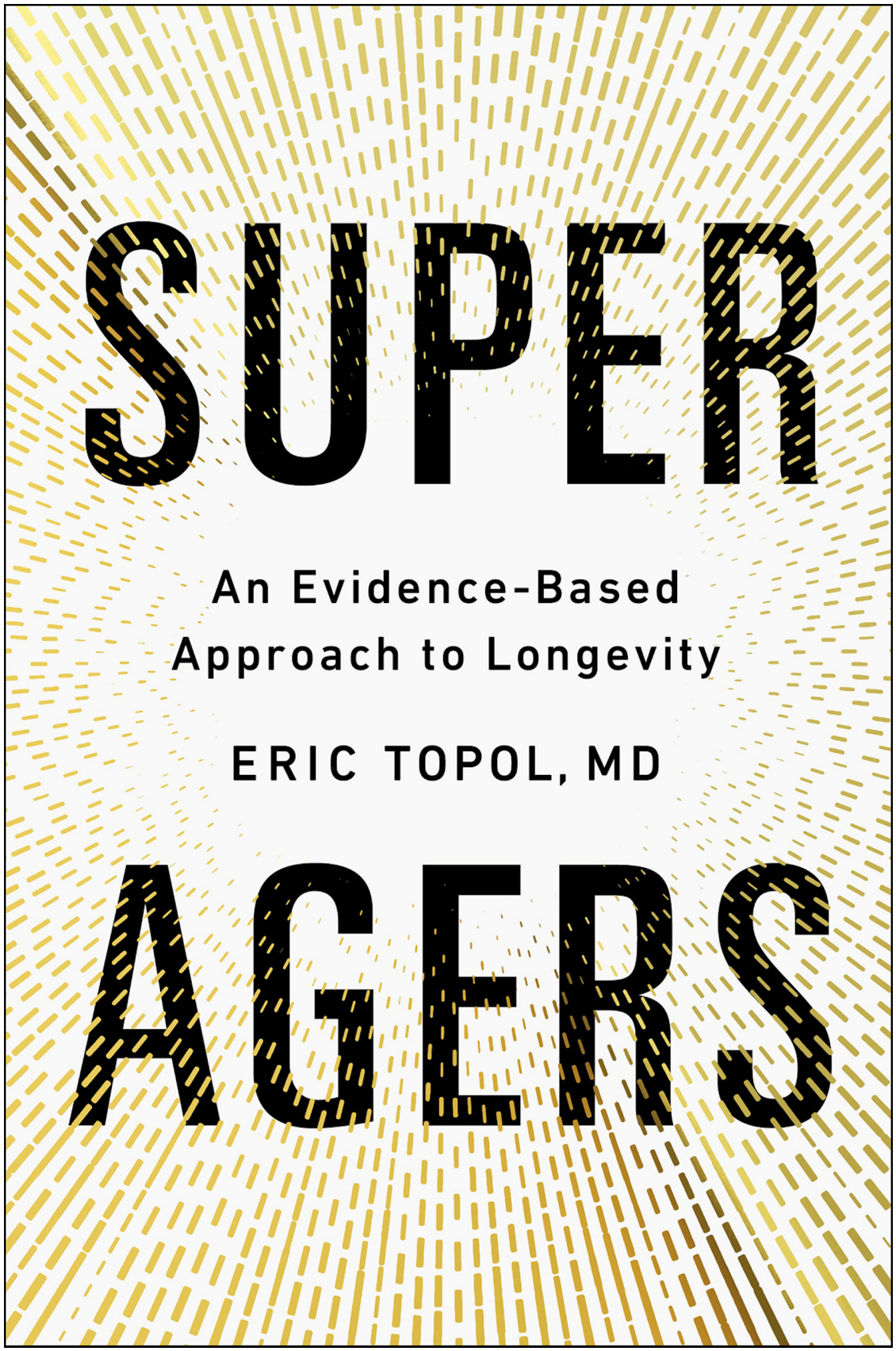
# SUPER AGERS

An Evidence-Based Approach to Longevity

ERIC TOPOL, MD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## Praise for *Super Agers*

"This eye-opening book reveals that the secret to longevity lies not in the miracle of antiaging pills, but in groundbreaking scientific advancements. Drawing on a vast body of research, Dr. Topol shows us how modern science is paving the path toward longer, healthier lives."

—Katalin Karikó, Nobel laureate and author of *Breaking Through*

"Eric Topol offers detailed insight into the factors that affect aging—and proof of AI's incredible potential to advance biomedical science, promote health, and extend longevity."

—Demis Hassabis, Nobel laureate and CEO of Google DeepMind

"A compelling and comprehensive guide to cutting-edge treatments that rejuvenate the body, prevent diseases, and enhance overall health."

—Steve Horvath, inventor of the Horvath aging clock

"Imagine a world where diseases once thought incurable are treated, where we are healthier for longer. This future is getting close thanks to a radical convergence of AI and bioscience. In this gripping, vitally important, and meticulously researched account, Eric Topol reveals the extraordinary breakthroughs that are transforming medicine—and our lives."

—Mustafa Suleyman, CEO of Microsoft AI

"In this highly readable and engaging tour de force, Eric Topol takes on a field full of overblown and premature claims, using his personal expertise as a physician to wade through the mass of conflicting evidence to separate fact from hype and pseudoscience, and shows us a path to healthy aging. People

contemplating how to make the most of their lives will benefit from reading this book."

—Venki Ramakrishnan, Nobel laureate and author of *Why We Die*

"*Super Agers* is a clear-eyed perspective on the drivers of aging from someone with a front-row seat to many of the most profound changes in healthcare and lifestyle over the past thirty years. Above all, this book is a call to action: for individuals to make changes to their lifestyle, for health professionals and scientists to explore emerging connections between different aspects of aging, and for society to make systemic changes that can benefit the health of everyone, particularly those at risk of being left behind in a new era of healthful aging."

—Feng Zhang, James and Patricia Poitras Professor of Neuroscience at MIT and core institute member of the Broad Institute

"An extraordinary book that offers a reality check as well as a blueprint for the future. Topol manages to navigate the science of aging, to distinguish it from the pseudoscience, and to provide readers an honest and measured sense of what we do and don't know. The result is a beautifully written journey into a world that is bound to change our lives."

—Siddhartha Mukherjee, author of *The Emperor of All Maladies*

"A beautifully written book packed with evidence and hope. Eric Topol's vision of how AI will reduce age-related diseases is truly inspiring."

—Geoffrey Hinton, Nobel laureate in physics for his contributions to AI

"*Super Agers* provides a remarkable insight into the aging process, controversies in the field, and opportunities to intervene to attenuate age-related pathologies. This is a must-read book for those interested in the revolution in our understanding of aging, providing inspiration and hope for future research efforts in this field."

—Charles Swanton, deputy clinical director, Francis Crick Institute, and University College London Hospitals

"A detailed, evidence-based, and optimistic dose of the truth. Dr. Topol's gift for expression of complex issues comes through again."

—Dr. Robert Califf, former commissioner of the FDA

"Eric Topol is not only a distinguished geneticist, cardiologist, and scientist, he is the most widely read and credible health 'futurist' I know. *Super Agers* is a highly readable and evidence-based digest of those astonishing advances in medicine, science, and technology that directly affect our lifespans. A landmark book."

—Abraham Verghese, author of *The Covenant of Water*

"*Super Agers* captures the extraordinary potential of today's biomedical revolution. Eric Topol's optimistic and inspiring vision makes this book an essential guide to the future of health."

—Jennifer Doudna, Nobel laureate and coinventor of CRISPR gene editing

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# SUPER AGERS

## An Evidence-Based Approach to Longevity

ERIC TOPOL, MD

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New York Amsterdam/Antwerp London

Toronto Sydney/Melbourne New Delhi

*To my inspirational force, my family: my wife, Susan; our children, Sarah and Evan; and our grandchildren, Julian, Isabella, and Seneca; and to all my patients over many decades who've given me the privilege of caring for and learning from them, and the impetus to make medicine better.*

"Every man desires to live long; but no man would be old."

—Jonathan Swift

"It is not enough for a great nation merely to have added new years to life—our objective must also be to add new life to those years."

—John F. Kennedy

"For the first time in the evolution of life on this planet, a species has developed the capacity to edit its own genetic makeup. That offers the potential of wondrous benefits."

—Walter Isaacson, *The Code Breaker*

# PART I

The Age of Health Span

# 1

# A Tale of Two Patients

**M**eet my patient Mrs. L. R. She's ninety-eight years young and has never suffered a day of serious illness in her long life. She was referred to me by her primary care physician to assess her heart condition because she had developed swelling in her legs, known as edema. When we first met in the clinic, I noted there was no accompanying family member, so I asked how she got to the medical center. She'd driven herself. I soon learned much more about this exceptionally vibrant, healthy lady who lives alone, has an extensive social network, and enjoys her solitude.

Her remarkable health span isn't shared by her family members. Her mother died at age fifty-nine; her father at sixty-four. Her two brothers died at ages forty-three and seventy-five. Three years prior to our meeting, her husband had died at age ninety-seven. He had also been quite healthy, with a similar health span profile in contrast to his parents and siblings, who all had chronic diseases and died decades younger. Following her husband's death, Mrs. L. R. got depressed and dropped thirty pounds. She lost her interest in her hobbies of painting and doing one-thousand-piece jigsaw puzzles. She did continue to play cards and Rummikub every week with a circle of eight women. One of these friends suggested she move from the house she'd lived in for decades to a senior residence apartment. The move led her to artists, new friends, and an extended social network. This all brought her back to her "old" self, fully restoring her passion for

her award-winning oil painting, and doing puzzles too. I met a woman brimming with optimism with a sunny disposition and an easy laugh.

The consultation needed to determine why she had some leg edema. Although she had no history of high blood pressure, her heart was thickened on my smartphone echocardiogram and her heart muscle function was well beyond the normal range. Her ejection fraction, the proportion of blood squeezed out of the main pumping chamber with each beat, was abnormally high. I diagnosed hypertrophic cardiomyopathy of the elderly, coincidentally a condition that I had described years before in the *New England Journal of Medicine*. Her heart had become stiffened and had difficulty relaxing. This accounted for the leg edema, the treating of which was straightforward. The leg edema cleared, and she has remained without symptoms.

Mrs. L. R. exemplifies healthy aging. She is the unusual individual who has escaped all the common age-related diseases, a resilience that defies what most of us expect from the human aging process. Her extreme health span and longevity, as well as her husband's, do not appear to be related to genetic makeup. Medical scientists don't understand it, but they would conclude, from all we know about the biology of aging and process of elimination, that it's stochastic, a random stroke of good fortune.

In marked contrast, let me briefly introduce another patient of mine whom I have followed for over thirty years, who is now also ninety-eight. At age seventy-five, following coronary artery bypass surgery at age sixty-two, Mr. R. P. presented with angina. At the time, I placed two stents in one of his bypass grafts that had developed blockages, which stem from buildup of atherosclerosis that limits blood flow. He later developed rapid atrial fibrillation resistant to medications and underwent two ablation procedures to maintain a normal heart rhythm. Many years subsequently, he had a shoulder replacement and sustained a small heart attack postoperatively. At age ninety-six, he was hospitalized with COVID pneumonia and, despite a prolonged hospitalization, did not develop respiratory failure and fully recovered. Mr. R. P. represents the triumphs of modern medicine. He had severe atherosclerotic cardiovascular disease, but with repeated restoration of blood supply to his heart and aggressive secondary prevention treatment, all was well. He embodies the medical progress we've made with age-

related diseases. What's exciting now is that we can accurately forecast heart disease as well as the other major diseases of aging in high-risk individuals many decades earlier and achieve primary prevention, or, at the very least, a marked delay in their appearance. Doctors can't promise to reverse or halt aging itself, but we can promise the second half of our lives can be much healthier than our forebears'. This is the type of health span extension that we will be seeing far more commonly in the future owing to the phenomenal advances in the five dimensions I highlight throughout this book.

## THE DIMENSIONS

### 1. Lifestyle+

Allow me to explain. We've known for a long time that lifestyle factors—diet, exercise, and sleep—play an important role in health span. But that knowledge has greatly expanded to what I call the dimension of "lifestyle+" because it now includes broadly defined environmental exposures. Being outdoors in nature, pollution, social determinants of health including loneliness, specifics of physical activity including strength, and precision dieting including time-restricted eating are all aspects of lifestyle+. The cliché "the devil is in the details" is particularly fitting.

### 2. Cells

The latest understanding of the nearly thirty-seven trillion cells in our body offers an entirely new range of insights. Each week researchers are publishing new work involving molecular sequencing, determining the genetic material of hundreds of thousands to millions of single cells. We understand not only their workings across time and space but also how we can manipulate and rebuild them. This changes everything. T cells can be taken out of our body and engineered to substantially rev up our immune response to cancer, or alternatively to suppress a self-directed immune response. We are making that process faster, cheaper, and

simpler by transforming a person's own immune cells inside their body. We can take an organ from another species, a heart, say, edit the genes that provoke an immune response, and potentially solve the dearth of human donor organs. We can change a person's white blood cells into stem cells and then make, for instance, pancreatic cells that produce insulin. We can even grow cells in a dish into beating, multichamber hearts—or even brains. These organoids can preview how a person's brain will respond to a treatment for cancer or neurologic conditions.

## 3. Omics

Within our cells and body tissue there are many layers of biologic information that have become known collectively as omics. This term originated with the study of our genome, comprised of three billion DNA letters. The other principal layers include our RNA, proteins, epigenetics (the packaging of our genome), and microbiome. In aggregate, this rich set of biological data is critical to defining our uniqueness as individuals and lays the foundation for individualized medicine.

The advances in omic science are stunning. By sequencing our DNA, we can identify common and rare gene variants that tell us about risks of major diseases well beyond our family history. Detecting tumor DNA in the plasma portion of the blood, known as a "liquid biopsy," can enable early diagnosis and treatment of cancer; abnormal proteins or RNA can indicate the beginning of a neurodegenerative disease or the earliest sign of preeclampsia. A cluster of proteins in our plasma can tell us about the aging clock for each of our organs. The microbiome of our gut is busy sending signals to our brain and shaping our immune response.

## 4. Artificial Intelligence

Artificial intelligence (AI) is starting to play a pivotal role in preventing age-related diseases. We're seeing how—across all dimensions—it can precisely

determine a person's risk for specific conditions and provide actionable steps and interactive coaching. Multimodal AI integrates layers of data—electronic health records, labs, images, omics, exposure to pollution, social determinants of health, and state-of-the-art medical knowledge—to build personal medical forecasts. Virtual medical coaches have been a fantasy of primary disease prevention for decades, though it is still not yet a reality. However, Thrive AI Health, a joint initiative between Open AI and Thrive Global, may be on the way to making that dream come true.

## 5. Drugs/Vaccines

New drugs and vaccines are being rapidly developed because we can now predict the atomic shape of more than two hundred million proteins. You're probably familiar with generative AI's propensity to hallucinate. But that's a good thing when you exploit this bug/feature to discover new proteins that don't exist in nature and may fulfill big unmet needs in medicine. Guess where Ozempic and Wegovy came from. AI didn't invent these man-made peptides out of thin air; their discovery was based on the naturally occurring ones that circulate at low levels in our bloodstream. These and other glucagon-like peptide-1 (GLP-1) drugs have far exceeded all expectations beyond the treatment of obesity and diabetes, improving the outcomes of heart attacks, strokes, heart failure, liver, and kidney disease—and more to come. New technology developed for COVID vaccines is now being successfully applied to a broad range of diseases, including cardiovascular and cancer. We now have drugs that take immune therapy against cancer to a new level, combining highly targeted chemotherapy to avoid noxious side effects. Vaccines to supercharge our immune response against cancer are taking hold, and the flip side, to block the immune system in autoimmune diseases. For the first time, after failing for many decades, we are seeing drugs for neurodegenerative diseases.

As shown in the graph below; all five dimensions interact with one another. Our lifestyle factors influence our microbiome and cells. Our response to drugs and vaccines is modulated by our genomics and cells, and discovery of new drugs

has been enhanced by gene variants and by AI. We've learned how to engineer our cells to become living drugs (fig. 1.1).

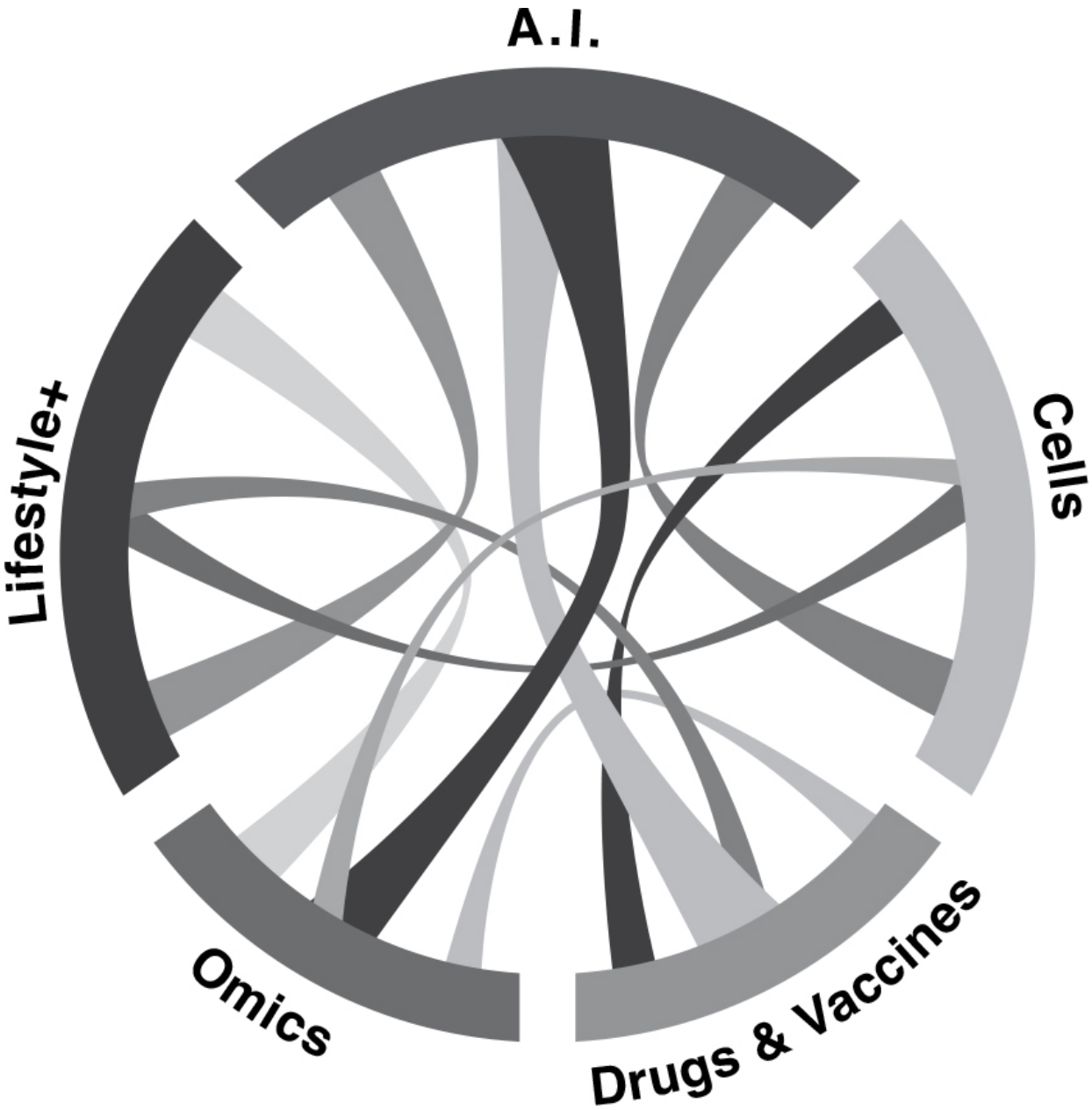


***Figure 1.1.*** The five dimensions and their interactions Description 1

## THE DRIVING FORCE

This multidimensional revolution in health span has been powered by a convergence of breakthroughs in life science and information technology. It has taken more than twenty years from the sequencing of the human genome, ten years from discovery of CRISPR genome editing, thirty years for mRNA and nanoparticle development, and many decades to get to large language AI models. Cumulatively, hundreds of years of dogged perseverance have put us in an enviable position of being able to reset our human health span expectations.

I ask you to bear with me. This book is evidence based. In some chapters the technical information is quite dense on the page. We are talking about life and death, and I know many readers feel an urgent need for the level of detail I offer. If nothing else, it will help families discuss options with their doctors. But it's my belief that these biomedical details build deep insights into our human potential.

# 2

# It's in Your Genes?

For many years, my colleagues and I have been fascinated by individuals like Mrs. L. R. who are so lucky to be so resilient to diseases. In 2008, we set up a research project called "Wellderly," to study people who were at least eighty years old and had never been sick or had a chronic illness. It took almost six years for us at Scripps Research to find fourteen hundred people who fit this extreme definition of healthy aging and consented to be participants, which meant they would provide a blood sample to have all three billion letters of their genome sequenced. We hypothesized that something in their genes would account for how these folks had such exceptional health span. I don't mean lifespan or longevity. Those measure the total number of years a person lives, whereas health span is the number of years lived in optimal health, without impairment due to disease or disability.

It turned out we were wrong. Despite the arduous and expensive task of sequencing and interpreting whole genomes several years ago, there wasn't much in their DNA to illuminate the basis for healthy aging. Their genetic risk markers for Alzheimer's and heart disease were only marginally smaller than they are for the rest of us. In contrast with the lack of novel genomic findings, this group of people, with an average age of eighty-four years, were obviously thinner, by almost thirty pounds, exercised more, and had more education than the general population of advanced-aged Americans. The research nurse who

enrolled and interviewed the participants[I] found the Wellderly to be remarkably upbeat people. Many had social interactions such as bridge clubs, dance, and a circle of friends; many were community volunteers. Well into their nineties, some were so busy it was hard to get an appointment to get them enrolled. Each had their own notion for why they were so healthy, despite that some were still smoking cigarettes—up to two packs a day. While our multiyear study failed to demystify the role of our DNA in attaining the most long-lived health span, it opened our minds to other factors.

Distinct from our Wellderly group of healthy aging outliers are most people; let's call them the Illderly. In the United States, 60 percent of adults (all ages eighteen and older) have at least one chronic disease, and 40 percent have two or more. Among those age sixty-five and older, 80 percent have two or more chronic diseases, 23 percent have three or more, and about 7 percent have five or more. If you or someone you know has a chronic condition, it is probably one of the big four: diabetes, heart disease, cancer, or some kind of neurodegeneration. Beyond these, chronic lung and kidney disease are high on the list.

Who wouldn't want to live a long life? Achieving longevity obsesses many of us. But living longer with chronic conditions such as Alzheimer's, a disabling stroke, or marked frailty doesn't seem all that ideal. What we really want is for the additional years of life to be essentially free from disease. The good news is that maximizing the years of living with intact health is becoming easier. This book is about how we can achieve our maximal health span, the shift from becoming Illderly to staying on the Wellderly path.

It can occur through two very different paths: by preventing or delaying age-related diseases or by slowing the aging process. The former is building on where we are now and for which we'll be making considerable headway in the near term. The latter, changing aging per se, is a more formidable challenge. Mrs. L. R. escaped the major chronic diseases, which only 19 percent of over four hundred centenarians (aged 97–119 years) managed to do in the New England Centenarian Study. The majority in that study, 81 percent, developed comorbidities, and were classified as either "survivors," having a diagnosis of age-related illness before age eighty years, or "delayers," diagnosed at age eighty and

beyond. Preventing or markedly delaying age-related diseases, thereby extending health span, is what this book is (nearly all) about.

## A Historic Convergence of Breakthroughs

The recognition of the immune system as a common mechanistic underpinning for chronic diseases, whether it be related to why they occur or to the untoward sequelae that they induce, is a historic turning point (fig. 2.1). These diseases—heart, cancer, neurodegenerative—take two or more decades to develop, giving us a longtime window of opportunity to prevent them. Atherosclerosis, which leads to heart attacks and strokes, endures, despite progress, as the number one cause of death and disability globally. It is due to inflammation in blood vessel walls, and all inflammation is generated by our immune system. Cancer isn't usually a killer unless it spreads, and our immune system can stop that from happening. If there weren't inflammation in the brain, it would be difficult for conditions like Alzheimer's or Parkinson's to take hold. When our immune system engages in self-directed attack on our body tissue, be it the nervous system in multiple sclerosis, or joints in rheumatoid arthritis, it becomes the foundation for autoimmune conditions. Dysfunctionality in our immune response is a principal driver of accelerated aging. Only in recent years have we begun to develop tools to handle the delicate modulating of our immune and inflammatory response. It's like a Goldilocks story whereby too little is not good and too much is bad, so we must get it just right—and target the precise tissue in the specific patient under care. Happily, the science of fine-tuning this pathway is rapidly advancing.

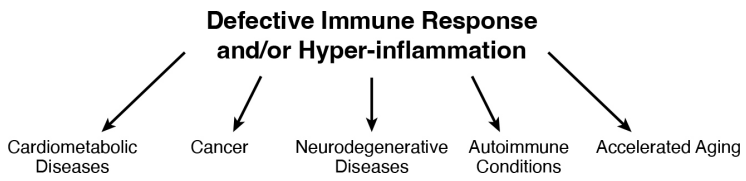


***Figure 2.1.*** The common thread underlying major diseases and accelerating aging Description 2

There are other pathways. Transplanting feces between people started to be pursued as a treatment in the 1950s, but only in recent years has there been unequivocal proof of its lifesaving capacity, leading to its FDA approval in 2023 to prevent recurrent *Clostridium difficile* infection, which can result from antibiotics changing the balance of bacterial species on the gastrointestinal tract. Fecal microbial transplant has a "yuck factor," obviously. It hasn't been easy either. But now "crapsule" pills have been produced and are in clinical trials, not only to make it easier but also to extend its use for managing various conditions such as cancer, gastrointestinal diseases, and diabetes. The idea of "CRISPR-ing" or editing the gut microbiome is being explored too.

Our immune white blood cells designated as T, which derive their name from our thymus gland's role in training them, are fundamental for fighting infections, differentiating between our own proteins and foreign proteins, and protecting us from diseases, such as cancer. T cell engineering, with or without genome editing, is emerging as a treatment for "liquid" tumors, blood diseases like leukemia, and is in early testing for hard-to-treat solid tumors, like pancreatic cancer. While it was previously considered far too expensive and impractical, the "off the shelf" potential has altered that perception.

The reversal of scarring (fibrosis) that occurs in many organs, previously considered as irrevocable, is being actively pursued in clinical trials with engineered T cells, known as chimeric antigen receptor (CAR-T) treatment, and new drugs, one of which was discovered using generative AI. CAR-T directed against fibrosis was used in mice to restore heart function. It was also used to achieve long-term remission of asthma and in the experimental model of multiple sclerosis to deplete a specific type of T cell population that attacks the body's cells. This groundbreaking work is not just in animal models: a single shot of engineered T cells has achieved remissions, without the need for immunosuppression therapy, in patients with lupus and other autoimmune conditions.

In the short term, we're going to get much smarter about individualized, optimal diets, with a $189 million investment by the National Institutes of Health (NIH), building on prior knowledge that each of us responds to food quite differently. Here the use of AI to help develop algorithms for what I call

Diet 2.0 is likely to represent a challenge to the old food pyramids and one-size-fits-all food recommendations—as if all human beings are the same.

With the astounding effectiveness of human papilloma virus vaccines to prevent cervical cancer, and COVID vaccines for preventing hospitalizations and deaths during the pandemic, there is keen interest to build on these successes. When it comes to empowering the immune response against cancer, there have been multiple reports that a vaccine directed to a person's cancer cell proteins, known as neoantigens, can increase successful treatment beyond the existing drugs known as checkpoint inhibitors. Ultimately, there will be cancer vaccines produced to prevent specific cancers in people at high risk or show the earliest molecular signs of onset (such as via a liquid biopsy), but these will require a jump from current efforts that are directed at augmenting the immune response in people who have already developed cancer.

Well before we get cancer vaccines, we will see a shakeup in how we do cancer screening. Today, this is largely based on age as the dominant factor, which means if you are in *this* age group, get *that* screening. But only 14 percent of cancers in the United States are detected via mass screening, an expensive and inefficient method, which also induces considerable anxiety due to a preponderance of false positives. We're also seeing a significant rise in cancer among young people, such as colon cancer in people in their twenties, which is well below the age threshold for screening. With the help of AI analysis of multiple layers of data from a person's health records, we can identify people at increased risk, irrespective of age. There is no need to screen people who have no—or extremely low—risk of cancer. Accurate prediction of high-risk status for major conditions like cancer and Alzheimer's that we can act on means we can live longer, healthy lives. Just integrating the information from our genes with our gut microbiome has substantially increased our medical forecasting capability across common chronic diseases.

We're already seeing preclinical evidence of vaccines putting the immune system in a kind of low gear that will likely be very useful for preventing or controlling autoimmune diseases. This approach applies to a wide range across type 1 diabetes, multiple sclerosis, and inflammatory bowel disease. Creating drugs and finding vaccines for prevention of Alzheimer's disease and other

neurodegenerative conditions have been the most vexing challenges, supplying a graveyard of hundreds of failed attempts. Even the ones that have recently garnered FDA approval have modest efficacy, at best. The relentless pursuit of this vital objective, especially with accelerated AI drug discovery, will someday lead to powerful interventions to block neuroinflammation and destruction of brain tissue. CRISPR genome editing, which has been approved for conditions that were previously untreatable but can now be cured, may help. CRISPR stands for clustered regularly interspaced short palindromic repeats, a certain pattern in our genetic code we hadn't noticed at all until 1987. The CRISPR pioneer and Nobel Laureate Jennifer Doudna posed this intriguing question: "Could we protect people who have a genetic susceptibility to Alzheimer's from getting it by using CRISPR to alter the genes that might be causing that predisposition, and doing it early, before somebody is already kind of in the throes of dementia?"

Over the decades, I've had the opportunity to do patient-oriented research for advances in biotechnology in large randomized trials, novel monoclonal antibodies, biosensors, genome sequencing, and genome editing of stem cells. While I've long been a skeptic that we'd *ever* see antiaging drugs, as opposed to drugs that treat specific diseases that mostly afflict those in later life, I've changed my mind. From the billions of dollars of investment in advancing the science of aging and start-up companies with top-flight scientists, I've become convinced that we're in the early stages of realizing that big audacious goal. It's an important reason I thought writing this book was warranted. Unfortunately, a lot of the hype right now is misplaced, not aligned with evidence in people, and can put the progress and the field at risk. Well before such systemic interventions against aging itself kick in, we'll be making major advances chipping away at the prevention of age-related diseases. That's the realistic and attainable counter to aging that is starting to blossom.

Let me emphasize up front that certain points of progress I mention in the chapters ahead may seem far off, as much as fifteen years away. However, keep in mind that I'm referring to the time when these changes will likely be incorporated into medical practice. That doesn't mean you wouldn't have the opportunity to take advantage of some of these now, which I'll identify, such as

upending your cancer screening plan, using AI to help make a diagnosis or as a second opinion, or manipulating your gut microbiome. Furthermore, your knowledge about many of the potential health span expanders now will raise awareness when they can be available to you or your family, even when they are in research protocols, well before their general acceptance.

## OBSTACLES

Health inequities across society are a profound problem in the United States, no less globally. If health span expanders are only accessible to the wealthy, then all they will do is make those inequalities worse. The lack of universal health care in the United States, unique among high-income countries, is holding us back. Genome editing for sickle cell anemia or GLP-1 drugs for obesity come at a high cost, making them less available to the people who can benefit the most. Efforts to improve health span for everyone must prioritize reducing and ultimately eradicating these gaps. We're beginning to see dedicated efforts to deliver these technologies to the underrepresented, such as mental health apps, AI diagnosis of retina disease in diabetics, or accelerated AI adoption for smartphone ultrasound imaging in low- and middle-income countries. Addressing narrow cultural biases that are embedded in AI models requires far more attention.

I reference results from the UK Biobank study of 502,000 participants many times in this book. With the participants' genome sequence, electronic health records, medical scans, and extended follow-up now approaching twenty years, the study has yielded seminal new insights for promoting health span. However, it is not a diverse population, with 94 percent of the participants of white ethnicity. We must have such resources that are multiancestry, and the All of Us initiative in the United States, the largest investment for a medical research program in our history, is pulling that together. I'm pleased to be a significant contributor to that effort.

Medical researchers face unnecessary delays in getting protocols approved and fulfilling enrollment targets, which adds up to great expense. There has been much effort to foster open science and broad collaboration, but we still have far

to go. Generative AI firms protect their most important and valuable models, which is understandable, but this is a serious obstacle to health care developments.

We saw how Operation Warp Speed, with a nominal investment of $12 billion in 2020, markedly accelerated the mass production and validation of COVID vaccines within ten months from the time the SARS-CoV-2 virus genome was first genetically sequenced. That investment was small compared with the increase of morbidity and loss of lives that would have occurred were highly effective vaccines not rapidly available. This was unprecedented in two ways: first, how fast a vaccine was produced and shown to be highly effective, which on average takes eight to ten years; and second, that the government invested to such an extent in a public-private industry partnership. Surely expanding the human health span is as worthwhile as halting the growth of one killer virus?

Compelling proof from medical research is difficult to come by without substantial dedicated funding. That validation is essential for acceptance in the medical community—doctors themselves must trust the evidence and be willing to change medical practice. Historically, the medical community has been resistant to change, especially when it involves ceding more responsibility to patients. The resistance to change is amplified by the need for regulatory approvals and the challenge of coming up with new reimbursement paths. These barriers add up to years lost in contradistinction to the number of years of health span that we could gain.

We have preliminary data to get us to some of the most far-reaching objectives of preventing serious age-related diseases. You may have felt, like I have, that you were dealt "bad genes" from your parents. For me, it was a father who went blind from autoimmune diabetes by age forty-nine and a mother who died of cancer in her early fifties. How can we be optimistic with such family histories? We now have more accurate ways to assess a person's risk for nearly all the major, common diseases, and these assessments will keep getting better. So too will our ability to prevent a dreaded condition from ever showing up or squashing it once it does. With the confluence of so many extraordinary biomedical and tech advances at once we are poised to move the needle and

extract many more high-quality, healthy life years. To transform health span as we know it. I'm eager to share with you my excitement, along with notes of concern, for how we'll augment the second half of our lives and come to be counted among the Wellderly. Soon, there will be many more people like Mrs. L. R. and Mr. R. P.

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I. The research nurse happened to be my daughter, Sarah Topol.

# 3

# Lifestyle+

Biomedical technologies and new expertise are transforming how we think about the second half of our lives, but we need to look first at the category of new knowledge that might sound a little old-fashioned. In part to dispel that impression, I've added "+" to the word lifestyle.

When we get into discussions of "healthy lifestyle," it usually refers to diet, exercise, sleep, and intake of alcohol, coffee, and tobacco. My much broader definition, lifestyle+, adds environmental conditions such as exposure to toxins including air pollution, microplastics, forever chemicals, socioeconomic status, loneliness, and social isolation. Thinking about diet must now include consideration of ultra-processed food, time-restricted eating, and the optimum amount of daily protein for you specifically. Exercise means more than aerobic fitness; it includes good posture, resistance weight training, and that which maintains your sense of balance along with more standard notions. The reason to address this dimension first is that many more healthy years can be added to our lives without fancy, expensive technology.

The studies that have been done on diet, exercise, and sleep are predominantly observational studies from large cohorts. Why? It is extremely difficult to randomly assign a lifestyle to a person and expect compliance for many years going forward. Instead, we observe "real-life" statistics in the wild rather than contrived experimentally focused studies. But these large real-life cohorts rely on

imprecise data, such as food diaries or self-reported memory for nutritional intake, physical activity, or sleep. Nonetheless, collectively they comprise an enormous body of evidence that can illuminate an "association" when there is a consistent pattern of benefit or harm, often with respect to magnitude of effect, across multiple reports. This does not establish a cause-and-effect relationship but merely a link that is supported by analytics adjusting for any confounding factors. The problem with such adjustments is that even the cumulative, small effects of confounding factors can be important, and some factors are simply overlooked. Many such studies suggest there is an effect on "all-cause mortality," meaning it doesn't matter what exactly a person died of—their heart condition, cancer, or some other disease. Nevertheless, all-cause mortality numbers are meaningful because they suggest there are real negative health effects via yet undiscovered pathways. Evidence from randomized trials, which are typically much smaller with more limited follow-up, deserve extra credence because they can provide a causal relationship about the intervention.

## DIET

The aphorisms "You are what you eat," which dates to 1826, and "Let food be thy medicine and medicine be thy food," misattributed to Hippocrates, reflect the long-standing belief in the vital importance of diet. A systematic assessment across 195 countries concluded that a poor diet is linked to 22 percent of all deaths, which accounts for more deaths around the world than tobacco, cancer, hypertension, or any other medical condition or health risk. So, what is a healthy diet? There are over sixty thousand diet books on Amazon, yet the evidence remains thin for what constitutes the best healthy diet, no less the presumption that it should be the same for all people. The global diabesity epidemic has been fueled, at least in part, by our diet, but the often-overlooked heavy influence of Big Food, an oligopoly of multinational corporations, must be recognized.

## Ultra-Processed Foods

Big Food makes a lot of these. Of course, it is not just how much you eat but what you eat. Ultra-processed foods (UPFs) are like UFOs; they are alien, industrially produced, unnatural substances; they're not even food. I'm not going to name brands of foods, but they are not what you'd find in a standard home kitchen to make meals from. There are basically two major dimensions—chemical and physical—by which industrial processing does its damage. First, these foods and beverages contain additives and industrial ingredients. It's a long list, but here are some of the chemicals you should avoid or limit: coloring agents, sweeteners such as maltodextrin, high-fructose corn syrup, fruit juice concentrates, dextrose, lactose, artificial sweeteners, hydrogenated oils, palm oil, calcium propionate, soy protein isolate, modified starches such as maltitol, calcium caseinate, hydrolyzed beef gelatin, and emulsifiers such as soy lecithin, xanthan gum, guar gum, and diacetyl tartaric acid esters of mono- and diglycerides.

Second, the manufacture of these foods involves physical changes in texture and form—molding, extrusion, prefrying—to maximize digestibility and accelerate digestive tract absorption, which in turn produces spikes in blood glucose and insulin. That's the opposite of dietary fiber, which slows digestion, reduces glucose spikes, and does us a lot of good.

Extrusion cooking requires temperatures above 100°C and shear forces that "melt" or "extrudate" food that is then molded into chips, snack bars, cookies, breakfast cereals, packaged pizzas, chicken nuggets, and doughnuts. Contaminants from packaging with such substances as bisphenols, mineral oils, and phthalates are not recognized in an otherwise helpful classification system known as NOVA: Group 1-unprocessed, Group 2-processed culinary ingredients, Group 3-processed foods, and Group 4-ultra-processed.

| NOVA Food Classification | | | |
|---|---|---|---|
| **Unprocessed or Minimally Processed Foods** | **Processed Culinary Ingredients** | **Processed Foods** | **Ultra-Processed Foods** |

| Unprocessed or Minimally Processed Foods | Processed Culinary Ingredients | Processed Foods | Ultra-Processed Foods |
|---|---|---|---|
| Foods that did not undergo processing or underwent minimal processing technics, such as fractioning, grinding, pasteurization, and others | Obtained from minimally processed foods and used to season, cook, and create culinary dishes | Unprocessed or minimally processed foods or culinary dishes that have added processed culinary ingredients; necessarily industrialized | Food products derived from foods or parts of foods, being added cosmetic food additives not used in culinary |

Adapted from https://www.theurbanco-op.ie/blogs/nova-food-classification

In a much-referenced randomized trial by Kevin Hall and colleagues at the inpatient NIH Clinical Center, two groups were given either unprocessed foods or ultra-processed foods for fourteen days (fig. 3.1). Participants could eat as much as they desired. It is no surprise which group ate an extra five hundred calories a day, mostly of carbohydrate and fat, with a significant change in weight gain. The explanation for this overeating of ultra-processed food likely involves the disruption of gut-brain signals that unprocessed food conveys to the brain.

Eating ultra-processed food is associated with a markedly heightened risk of cardiovascular and metabolic diseases. These foods induce abnormal lipid levels, insulin resistance, and systemic inflammation. Furthermore, a diet rich in these foods is linked to 80 percent elevated risk of metabolic syndrome, 40 percent higher risk of type 2 diabetes, 23 percent increased risk of hypertension, 55 percent risk of obesity, and 66 percent risk of cardiovascular death. Increasing UPF in the diet leads to higher risk of cardiovascular, coronary artery, and cerebrovascular disease. Among older adults, a mere 10 percent increase of UPF intake is associated with a 16 percent increased risk of cognitive impairment. Regular consumption of ultra-processed red meat, such as bologna, bacon, sausage, and hot dogs, has been associated with a 14 percent higher risk of dementia. There is a remarkable dose-response curve.

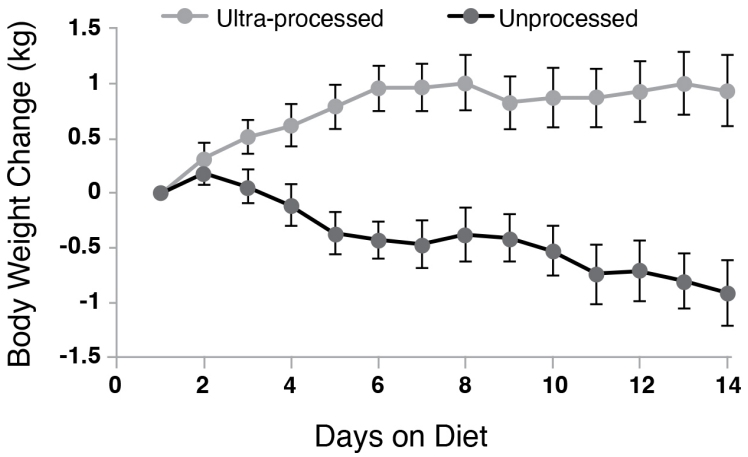



***Figure 3.1.*** A randomized trial showed that participants who ate an ultra-processed food diet gained weight compared with those who consumed an unprocessed food diet and lost weight. Adapted from Kevin Hall et al., "Ultra-processed diets cause excess calorie intake and weight gain: An inpatient randomized controlled trial of ad libitum food intake," *Cell Metabolism* 30, no. 1 (July 2019): 67–77 e3, https://doi.org/10.1016/j.cmet.2019.05.008. Description 3

It's not just a matter of cardiovascular or metabolic or cognitive disruption. UPFs are associated with fatty liver disease, most types of cancer, sleep disorders, inflammatory bowel disease, depression, and dementia. A 62 percent increase in all-cause mortality is linked to more than four servings per day of UPF. This was echoed by a comprehensive review of forty-five studies involving a thirty-year follow-up of about seventy-five thousand women and nearly forty thousand men.

My friend Chris van Tulleken, a British physician-scientist who wrote the outstanding book *Ultra-Processed People*, conducted an experiment on himself. He increased his diet from 20 percent to 80 percent ultra-processed food for a month, with extensive baseline and follow-up assessments, including MRI brain scan and lab tests for lipids and inflammation. He gained fifteen pounds. As he put it to me, "There were enormous changes in connectivity between the habit, automatic behavior bits at the back in the cerebellum and the reward addiction bits in the middle of the limbic system and associated brain regions." Chris's hunger hormones went sky high, such as a fivefold rise in leptin, and there was a doubling of his C-reactive protein that tracks with body-wide inflammation. Being hungry and swollen doesn't sound fun.

Chris and his identical twin have had whole genome sequencing that indicated they have many genomic variants that predispose them to obesity. However, when his twin brother, Xand, followed the same diet of ultra-processed foods, he

got forty-four pounds heavier than Chris. That represented the biggest weight difference in the very large twin study conducted in the United Kingdom. Once again, it's not all in the genes.

For healthy aging, UPFs must be restricted in your diet to the lowest level possible.

That's why it's so important to read labels and select items without additives, without added or fake sugars, with as few ingredients as possible. If there's a health claim, beware. High UPFs are usually found in the middle aisles of grocery stores, which explains the helpful tip to do most shopping in the perimeter for fresh foods. When there's a question, consider using the Open Food Facts app, developed in France with the help of tens of thousands of volunteers providing information for about three million substances.

Why hasn't the United States joined other countries like Brazil, Israel, Belgium, Chile, and Uruguay in publishing guidelines for people not to eat UPFs? There could be demands to reformulate UPFs, but that's not happening. In the United States, the multinational corporations behind Big Food have a supersized chokehold influence on the Department of Agriculture. Big Food spends twice as much lobbying the government as the tobacco and alcohol industries combined. The *F* in FDA stands for Food, but there is little evidence that our regulatory body has true oversight and authority about potentially toxic constituents that we're eating. There's not even heightened UPF awareness led by the FDA or other public health agencies. In late 2023, the FDA proposed banning brominated vegetable oil, a food additive found in beverages for a century that was previously banned in Japan and Europe. Likewise, Red Dye No. 3 (found in candy), propylparaben (found in baked goods), and potassium bromate (found in packaged breads) have been banned by the European Union because they are all linked to serious health problems. In the United States, only California approved such a ban for all four of these additives, but that won't take effect until 2027. Recall how it took the United States multiple decades after Europe to ban trans-fat foods, despite unequivocal evidence of cardiovascular harm.

Over time, it is quite likely that ultra-processed foods will be regarded as akin to cigarettes; public awareness of their dangers were suppressed for decades.

Realistically, UPFs will not be banned, but there could be regulations enacted to reduce their toxicity and to enforce conspicuous labeling on all foods and beverages that spotlight their risks. Such labeling worked well in terms of public health for cigarettes.

## Sweeteners

While sugar activates our dopaminergic reward circuits and provides hedonic value, too much of a good thing is clearly bad. The biggest source of added sugar in diet is from sugar-sweetened beverages. There are fifty grams of simple sugar in a can of Coca-Cola, and forty-four grams in a Starbucks Frappuccino. As seen in a large cohort of more than thirteen thousand people age forty-five and older, followed for six years, consumption of high sugary beverages, including fruit juices, was associated with a 24 percent increased all-cause mortality. This finding was independently replicated and extended by several other studies, in much larger cohorts and with longer follow-up, for both increased cardiovascular and cancer-related mortality. Notably, atrial fibrillation increased more than threefold during a ten-year follow-up among the two hundred thousand participants who drank more than two liters per week. The consistency of all the reports indicate that consumption of sugary beverages should be limited, and this includes moderating the intake of fruit juices for children. The evidence for fueling the burden of oral diseases including cancers and dental caries is also clear.

The story of nonnutritive, artificial sweeteners is a bit more complicated, with conflicting reports regarding the many nonsugar substances. A comprehensive review of all the available data from randomized and observational studies found a lack of compelling evidence for associated risk. In contrast, another large cohort of more than one hundred thousand people found a direct association between consuming such sweeteners, particularly aspartame, acesulfame potassium, and sucralose, with cardiovascular and cerebrovascular disease risk. But that was not the case when different artificial sweeteners were examined separately through a randomized trial. In 120 healthy adults given saccharin, sucralose, aspartame, and stevia, versus controls for two weeks, the first two sweeteners induced impaired glucose regulation (also known as glycemic response), and all four were associated

with changes in the oral and gut microbiome. Previous work has shown that such alteration of the gut microbiome is tied to the abnormal glycemic response. Overall, the data for artificial sweeteners is unfavorable, although it is not nearly as worrisome as high sugar consumption. Certain sweeteners, such as stevia, appear to be less concerning than others.

## Salt

While the magnitude of the effect is debated, the link of sodium intake to hypertension is clear. A review from the Cochrane Library of all available data from 195 interventions studies that compared low- versus high-sodium diets concluded the effect of restriction was small, with a decrease in mean blood pressure of only 0.4 mm Hg, slightly greater among Black and Asian participants. In treating innumerable patients for hypertension over a few decades, I've seen that reduction of dietary sodium can help reduce blood pressure, as confirmed in many studies, but the impact varies considerably and may be small in many. An extensive analysis of sodium intake suggested moderate consumption (1–2 teaspoons of salt; 2 g of sodium = 5 g of salt) was not a problem. But increased cardiovascular risk became obvious at levels of more than 5 grams of sodium per day. Recommendations from the American Heart Association, World Health Organization, and European Society of Cardiology vary considerably, from 1.5 grams to 2.25 grams per day. By the same metric, the average American's intake is about 3.5 grams!

The risks from dietary sodium are not confined to high blood pressure. Multiple experimental model studies support high salt diet's reduction of blood flow to the brain through dysfunction of the blood vessel wall (endothelium) and potential risk of cognitive impairment. The finding that people who consume the least amount of sodium have the lowest risk of cardiovascular disease has been backed up by other studies.

The best general advice for people with hypertension is to avoid or limit adding salt to foods, to keep an eye on overall intake by paying attention to food labels, and, when no kidney disease is present, to consider using a potassium chloride salt substitute. The evidence for salt substitute from a randomized trial

of more than six hundred participants with normal blood pressure, average over seventy years of age, is encouraging—there was a 40 percent reduction of hypertension. A systematic analysis of the evidence for long-term salt substitution supported reduction of all-cause and cardiovascular mortality. The lower, the better is easy to say. I acknowledge Sir George Pickering's perspective from 1961: "The rigid low-sodium diet is insipid, unappetizing, monotonous, unacceptable, and intolerable. To stay on it requires the asceticism of a religious zealot."

## Carbs, Protein, and Fat

The emergence of the term *carbotoxicity* conveys the multiplicity of risks associated with excessive intake of carbohydrates. As with everything, moderation is the ticket: both low, less than 40 percent, and high, greater than 70 percent, daily caloric intake are associated with increased all-cause mortality. Much of the relationship can be affected by the type of carb, with good comprising resistant starch and other high-quality carbs in the form of dietary fiber. From 185 prospective studies, 58 randomized clinical trials, assessing dietary fiber of 25–30 grams per day, there was an association with a 15–30 percent reduced all-cause and cardiovascular mortality, type 2 diabetes, and colon cancer.

In contrast, low-quality carbs are fast-digesting, like the sugars we've covered. They also include potato products, or refined grains, which are a high glycemic load, raising insulin levels and predisposing to weight gain. A high glycemic index diet among more than 137,000 people ages thirty-five to seventy years on five continents was associated with more than a 25 percent increase in cardiovascular deaths. Good, unprocessed carbs of nonstarchy vegetables, legumes, fruits, and whole grains are the ones to give priority.

Much of the current guidance via the National Academies' Recommended Dietary Allowance (RDA) for protein intake is based on relatively scant evidence, mostly from short-term nitrogen balance studies in young adults, underestimating what is needed in older individuals and longer term. The recommended daily intake of protein is 0.8 gram per kilogram of weight, 11 percent of total energy. You can get your somewhat personalized Dietary Reference Intakes calculated; my US Department of Agriculture

recommendations are shown in the table below (vitamins and minerals are not listed). When I put in parameters for a much younger man, the output didn't change very much, and the estimated daily caloric needs is highly influenced by your input of "active" or "very active." The protein RDA is remarkably low, and that will not help prevent loss of muscle mass with age—by age eighty, the average person will lose eight kilograms of muscle from their peak. The evidence suggests that for older adults, higher protein intake is needed, but the optimal amount is unknown.

| My US Department of Agriculture Recommendations | |
|---|---|
| Body Mass Index (BMI) | 23.6 |
| Estimated Daily Caloric Needs | 3,544 kcal/day |

# MACRONUTRIENTS:

| Macronutrients | Recommended Intake Per Day |
|---|---|
| Carbohydrate | 399–576 g |
| Total Fiber | 30 g |
| Protein | 69 g |
| Fat | 79–138 g |
| Saturated Fatty Acids | As low as possible while consuming a nutritionally adequate diet |
| Trans Fatty Acids | As low as possible while consuming a nutritionally adequate diet |
| a-Linolenic Acid | 1.6 g |
| Linoleic Acid | 14 g |
| Dietary Cholesterol | As low as possible while consuming a nutritionally adequate diet |
| Total Water | 3.7 L (about 16 cups) |

(https://www.nal.usda.gov/human-nutrition-and-food-safety/dri-calculator)

Some doctors, like Peter Attia, have advocated for intake of one gram per body weight in pounds (not per kg), which has not been substantiated by evidence. Indeed, a study of high-protein diets in people and mice warned of the danger of promoting atherosclerosis through an increase in plasma leucine, an essential amino acid building block of proteins, and impaired cellular waste disposal (autophagy). The gut microbiome of people who consume a high-protein diet (more than 1.5 g/kg) has been shown to produce pro-inflammatory metabolites. While increasing your diet up to 1.2 gram/kilograms of protein is reasonable, it's the leucine-rich animal proteins that should be avoided. High-protein diets are intended to reduce the risk of sarcopenia—age-related loss of muscle mass and strength—which has been unequivocally demonstrated (in many studies, such as quantifying handgrip) is a well-established risk factor for reduced health span. We don't know that increasing protein in the diet with age titrates or negates that risk, which would require a randomized trial with multiyear follow-up that will likely never get done. (That's because randomized trials of diets are extremely hard to do at scale; also, it's difficult to rely on participants' long-term adherence and difficult to obtain financial support.) Furthermore, there's no information on food labels about the specific amino acids (nine of the twenty) in our diet that we require because we can't synthesize them—such as leucine, lysine, and methionine. Note that the recommendations above break down fats but not proteins. The hard truth is that we could be doing so much more to upgrade our knowledge of dietary protein intake that's not being done.

Like sugar, there is a dedicated gut-brain reward circuit for fat intake conveyed through the vagus nerve. Separately, the fat circuit encourages more caloric intake, but it is synergistic with the sugar reward circuit for promoting dopamine release and overeating. Single-cell studies identified that the vagal neurons involved in the circuit, when silenced, abolished fat intake preference. Just as we've seen with salt, carbs, and proteins, there's no shortage of uncertainty. Take dairy products, a source of saturated fat, for example. The guidance to steer us to low-fat milk and yogurt dates back to 1980 and has never been updated. A 2018 report in more than 130,000 people from twenty-one countries followed for nine years found that consumption of two or more servings of dairy daily was associated with 22 percent less cardiovascular disease and 17 percent less all-cause

mortality, with a greater reduction in those with greater whole fat dairy consumption. That same year a report from more than sixty thousand adults tracked for an average of nine years found a 29 percent reduction in type 2 diabetes related to greater dairy intake. Particularly yogurt (without sweeteners) and cheese (harder like cheddar or parmesan for slower absorption) have been most associated with better outcomes, which coincides with some current recommendations for three servings of dairy per day, with one or two made up of yogurt or cheese.

A running theme here is that the subtype of each macronutrient—carbs, protein, or fat—matters. The relationship to all-cause mortality demonstrated among more than 125,000 adults followed for up to thirty-two years is shown in figure 3.2. Note that the shift to unsaturated mono- or polyunsaturated fats is accompanied by more favorable longevity data. The shift from saturated fats to plant-based unsaturated fats was associated with substantial reduction of risk of cardiovascular and type 2 diabetes in four clinical trials.

In the influential *Science* magazine paper "Dietary Fat: From Foe to Friend?" by David Ludwig and colleagues, the answer depends on the quality of the fat, not so much the content level. The ketogenic diet, which relies on high fat, less than 10 percent carbs, and less than 20 percent protein, has been popular for weight loss. But it is also associated with higher cholesterol and cardiovascular risk, brain fog, flu-like illness (keto-flu), and promoting fatty liver disease. A randomized trial at the NIH Clinical Center compared a ketogenic diet (76 percent fat, mostly animal based) with a plant-based, low-fat diet with no limit on the amount of food eaten. Although it presented less glycemic load to the participants, the ketogenic diet led to about seven hundred calories more ingested per day over two weeks. So it is not surprising that the low-fat diet group lost more weight. This small, rigorous trial defied the expectation for the carbohydrate-insulin model, which shows higher insulin driving more hunger and food intake. A fascinating study in mice showed a high-fat-diet-induced mitochondrial dysfunction, and through mitochondrial RNA, this was transmitted via sperm to male offspring. That is a particularly unnerving reason to be concerned about the impact of high-fat diets.

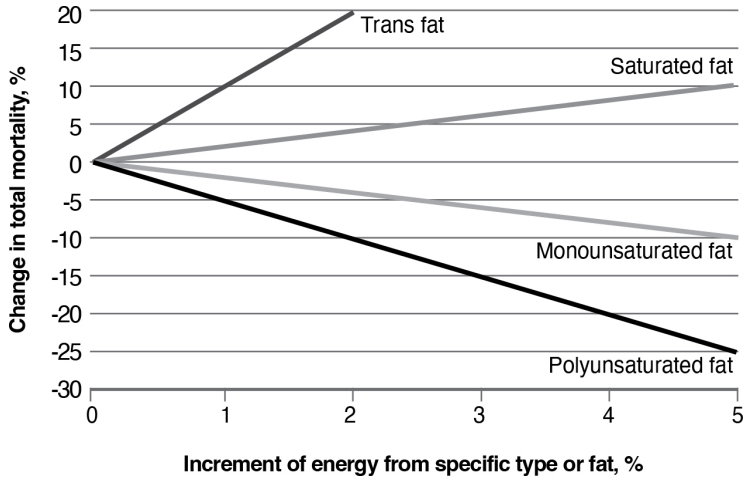



***Figure 3.2.*** Relationship of dietary fat sources and total mortality. Adapted from Dong Wang et al., "Association of specific dietary fats with total and cause-specific mortality," *JAMA Internal Medicine* 176, no. 8 (August 2016): 1134–45, https://doi.org/10.1001/jamainternmed.2016.2417. Description 4

## Caffeine

Coffee lovers enthusiastically received reports of up to a 30 percent reduced mortality, such as from more than 170,000 participants in the UK Biobank and another UK Biobank study supporting a dose response: the more coffee, the longer the person lived. However, most of the studies converge on about four cups per day linked with maximal benefit. A study of more than 520,000 people in ten countries in Europe followed for over sixteen years found a 12 percent reduction in all-cause mortality and 22 percent reduction for cardiovascular mortality. In three large cardiovascular cohort studies, increasing coffee intake was consistently associated with less heart failure. In well over two hundred meta-analyses of coffee and caffeine consumption, there was an association with less risk of breast, colon, endometrial, and prostate cancer; cardiovascular disease; Parkinson's disease; and type 2 diabetes. These are all observational studies that have a major caveat that the benefit could be linked to other factors, such as lifestyle, diet, and exercise, that might be contributing. We don't have a cause-and-effect relationship or unequivocal *proof* that coffee improves health outcomes.

Nonetheless, the overall good news on coffee and caffeine is complemented by studies looking at effects on the heart, particularly inducing rhythm disturbances

like premature ventricular contractions (PVCs), premature atrial contractions (PACs), or atrial fibrillation. For decades patients have been warned against coffee for fear of just this kind of heightened risk to heartbeat. A randomized trial showed no increase in PACs but did show some increase in PVCs. Nearly 450,000 participants in the UK Biobank did not show a higher risk of arrhythmias among coffee drinkers. In fact, among another large study of UK Biobank participants, for every cup of coffee increase, there was a 3 percent lower risk of a heart rhythm disturbance, including atrial fibrillation and ventricular tachycardia. This study also looked at genetic markers that affect caffeine metabolism, such that slower metabolism might be linked with more arrhythmias —but that wasn't the case.

The totality of the data support lack of hazard for coffee and caffeine, and the potential, albeit with some uncertainty, for some health benefits, even seen in some studies with decaffeinated coffee. If true, we don't know how coffee promotes health. Many hypotheses have been offered, such as promoting brown adipose tissue, and one based on the insulin sensitivity of coffee's polyphenol chlorogenic acid. Perhaps coffee works as an antioxidant, favorably modulates the gut microbiome, reduces inflammation, or facilitates DNA repair. This level of uncertainty about the causal connection is unsettling.

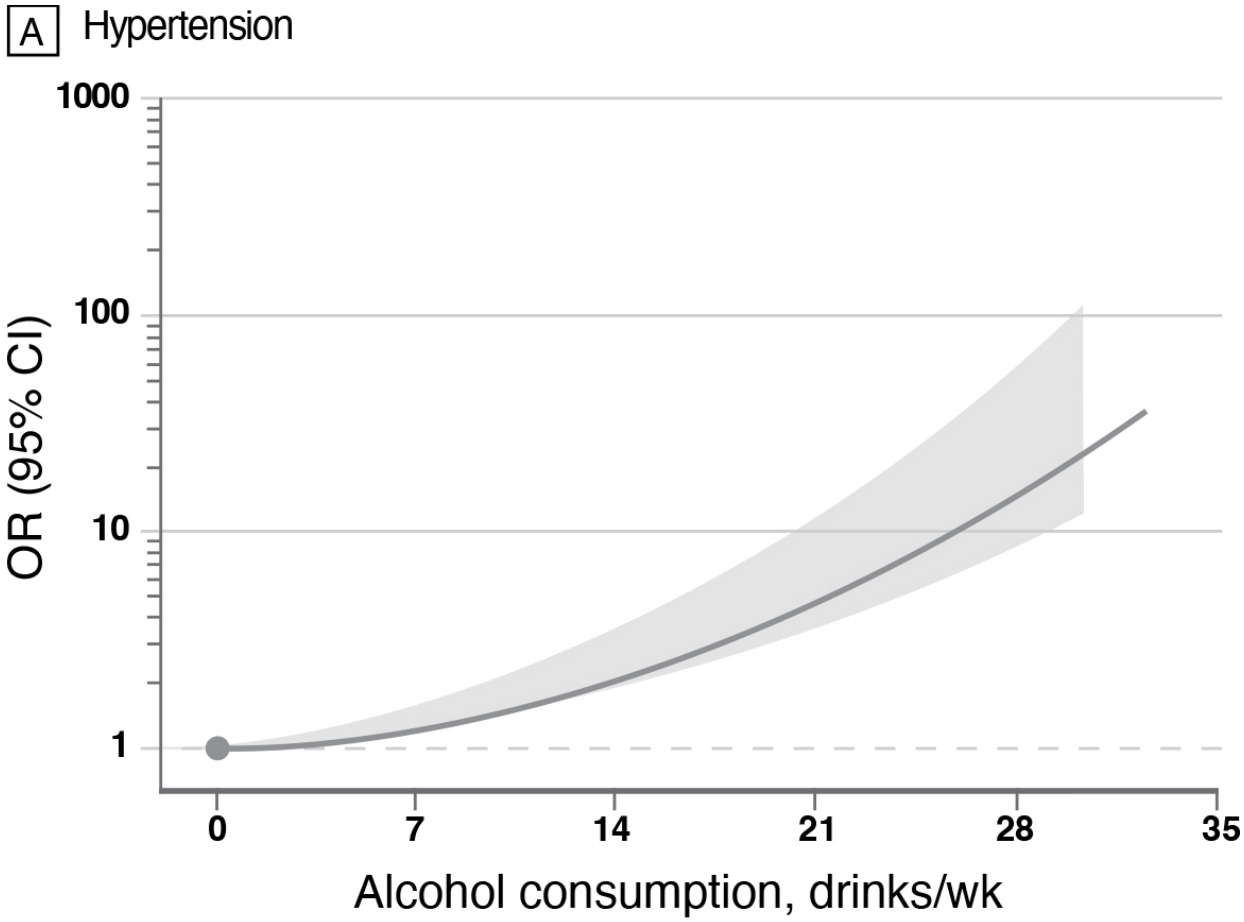
## Alcohol

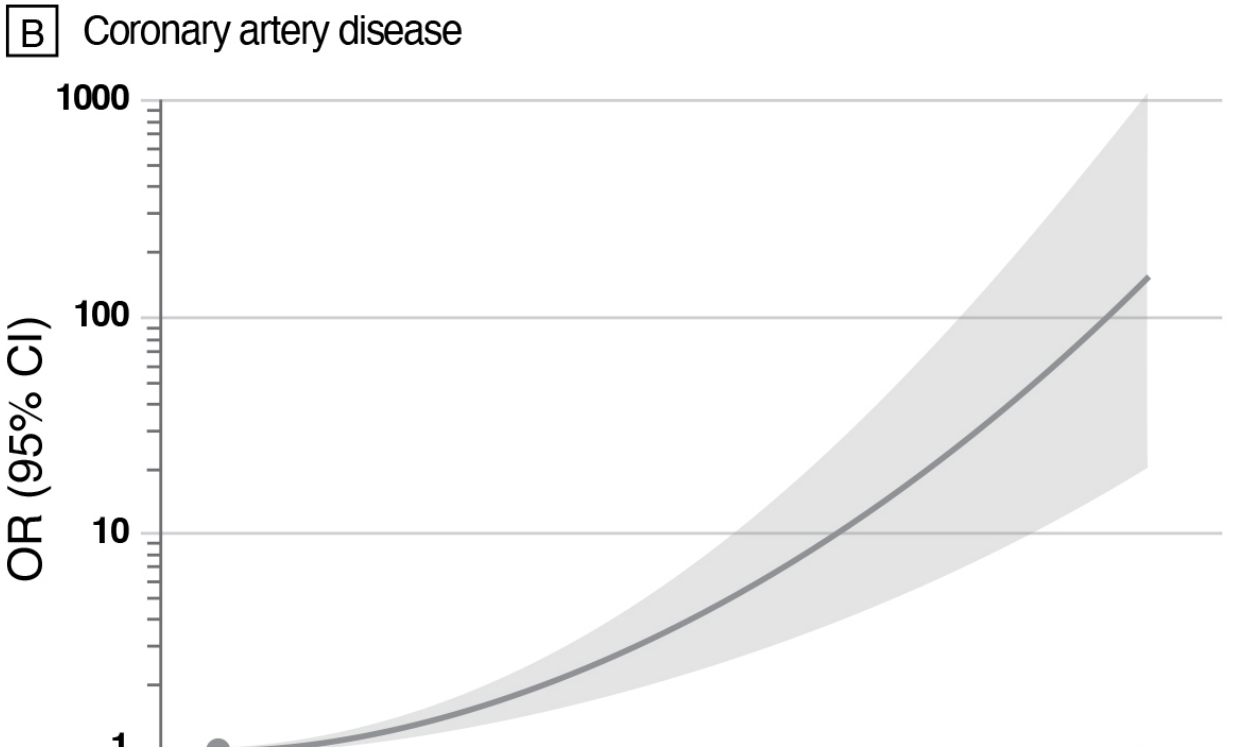
The profile we see in the evidence surrounding alcohol consumption is less blurry than for coffee and caffeine. Both moderate and heavy drinking does one no good. The long-standing myth about the benefits of red wine and the French connection was never adequately substantiated; instead, it's been refuted in an analysis of almost six hundred thousand people in eighty-three prospective studies. The International Agency for Research on Cancer classifies alcoholic beverages as carcinogenic, with a strong link to oral cavity and esophageal cancers established in multiple cohort and case-control studies. Ireland is mandating labels on alcohol products: "There is a direct link between alcohol and fatal cancers." Other countries, such as Canada, Norway, and Thailand, are beginning to follow suit. Most studies support a J-curve, with potential small benefit, both

for cardiovascular and cancer, with light intake (such as two drinks per week), which varies by age, but significant risk beyond this level of consumption. A dose-response relationship between alcohol intake and high blood pressure has also been noted. This was reinforced by a UK Biobank study that also factored in genetic predisposition (known as Mendelian randomization) to alcohol consumption. The evidence supports a causal relationship between alcohol consumption and two different types of cardiovascular disease (fig. 3.3). One study showed a small added risk at less than seven drinks per week, but an exponential rise with higher intake levels. Perhaps the best summary from the mixed data is that light alcohol intake is not a problem, but the risks quickly increase.

## Red Meat and Plant-Based Diets

I've grouped these together because they are at opposite ends of the spectrum for reduced versus increased risk of mortality and relative environmental impact. At one end is processed meats like hot dogs, bacon, and sausages that are linked to the highest risk, deemed carcinogenic by the World Health Organization, and their production leads to large amounts of greenhouse gas emissions. Not far away for increased associated mortality risk are unprocessed meats, pork and beef, which have been labeled "probably carcinogenic."


A
Hypertension
1000
100
10
1
OR (95% CI)
0
7
14
21
28
35
Alcohol consumption, drinks/wk


B
Coronary artery disease
1000
100
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OR (95% CI)

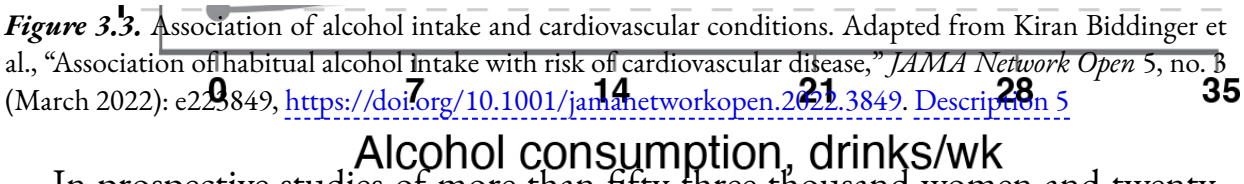
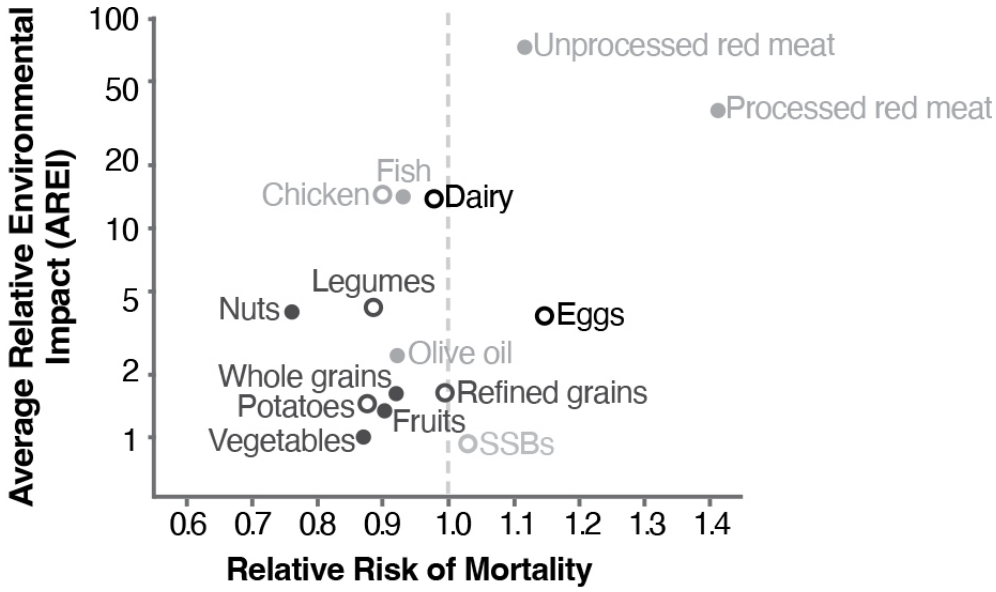



***Figure 3.3.*** Association of alcohol intake and cardiovascular conditions. Adapted from Kiran Biddinger et al., "Association of habitual alcohol intake with risk of cardiovascular disease," *JAMA Network Open* 5, no. 3 (March 2022): e223849, https://doi.org/10.1001/jamanetworkopen.2022.3849. Description 5

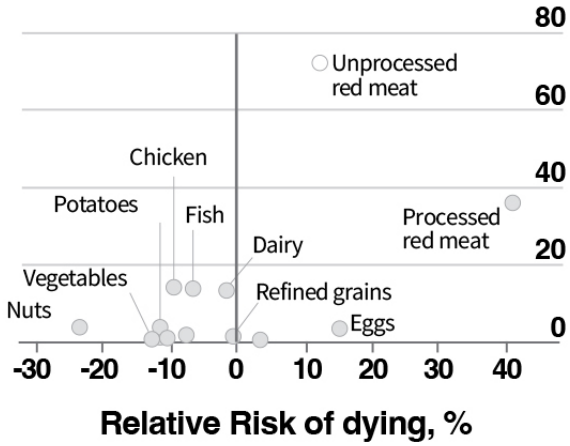
In prospective studies of more than fifty-three thousand women and twenty-seven thousand men, with an eight-year follow-up, there was a clear association with increased mortality (fig. 3.4). The environmental impact of the opposite poles represents a marked difference: a switch from high meat to a vegetarian diet would reduce greenhouse gases by 47 percent. But this twofer of putative benefit for health and the environment is not clear-cut. A systematic review of all the studies (nearly 120) did corroborate the increased risk of cancer for processed and unprocessed red meat, but concluded it was small and with low certainty. A similar study made the same point for cardiometabolic outcomes. It certainly isn't settled. A more recent study of nearly thirty thousand Americans followed for nineteen years concluded that both processed and unprocessed meat, but not poultry or fish, were linked to a small increased risk of all-cause mortality. There was a twist with respect to poultry: two or more servings of unprocessed or processed meat or poultry was associated with a 4 to 7 percent increased risk of developing cardiovascular disease.

In contrast, plant-based foods are plainly healthier. A summary of nine studies in more than three hundred thousand participants linked plant-based diets with 23 percent lowered risk of type 2 diabetes. A meta-analysis of thirty-two studies indicated substantial protection from all-cause mortality, cardiovascular deaths, and type 2 diabetes when processed meat diets were substituted by plant-based foods. Among more than seventy-two thousand participants in Japan, the intake of plant protein was linked to an 11 percent reduced risk of total and cardiovascular-related mortality. Compared with a ketogenic diet, a vegan diet enhanced the immune system responsiveness. Substitution of plant for animal protein for 3 percent of caloric intake was associated with 40 percent less cancer-related mortality. A systematic review added to the reduction of cancer risk linked to plant-based diets. Using DNA methylation markers, a way to assess epigenetic age, a plant-based diet slowed the pace of aging compared with an omnivore diet in a randomized trial of identical twins.



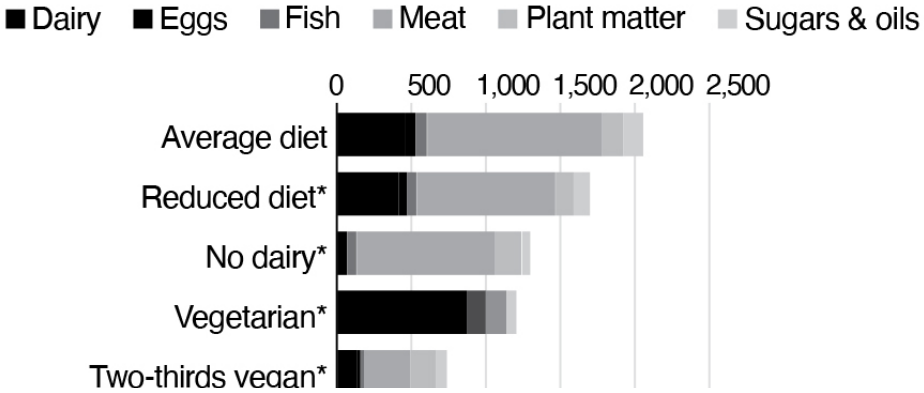

**Health and environmental impact of one extra serving per day**

Average relative environmental impact




**United States, greenhouse-gas footprint**

kg of $CO_2$ equivalent per person per year



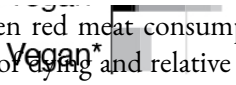



***Figure 3.4.*** Relationship between red meat consumption, mortality, and environmental impact. Risk of mortality (upper panel) and risk of dying and relative environmental impact (middle panel). Adapted from Michael Clark et al., "Multiple health and environmental impacts of foods," *Proceedings of the National Academy of Sciences of the USA* 116, no. 46 (November 2019): 23357–62, https://doi.org/10.1073/pnas.1906908116(lowerpanel); and "How much would giving up meat help the environment?," *Economist*, November 15, 2019: https://www.economist.com/graphic-detail/2019/11/15/how-much-would-giving-up-meat-help-the-environment? (middle and lower panels). Description 6

What about plant-based meat alternatives? They are high in sodium, higher in saturated fats compared with high-protein plant sources including lentils and beans, and can be high in heme, a component of hemoglobin containing iron, as in the case of the Impossible Burger, which has been associated with more risk of type 2 diabetes. But there's a lack of adequate studies. The bottom line is that the more red meat you eat, especially processed red meat, the less healthy you are likely to be. To date, the measured increase in cardiovascular and cancer risks are small but consistently point in the wrong direction.

## Good Food

Many studies have confirmed the beneficial effects of "healthy eating." Three large prospective studies with cumulatively more than 165,000 women and 43,000 men, followed up for thirty-two years, were consistent in showing a 15 to 20 percent lower cardiovascular risk linked to eating well. A lower risk of all-cause mortality was found in two of these prospective studies, with a dose relationship seen with cardiovascular, cancer, and neurodegenerative disease. For all these ailments, the higher the dose of healthy foods, the lower the risks. That's good, but what exactly does healthy eating mean?

We know it includes fruits and vegetables; legumes, such as lentils and garbanzo beans; whole grains; nuts and seeds; healthy fats, such as olive oil and avocados; and fatty fish, such as salmon and tuna, rich in omega-3 fatty acids. Consumption of olive oil, per se, has been tied to about a 20 percent lower all-cause mortality, along with reduction of cardiovascular-, cancer-, and neurodegenerative-related deaths. Among more than ninety-two thousand participants followed for twenty-eight years, there was a significant lower risk of

dementia: 28 percent less risk if a person consumed 7 grams (half a tablespoon) a day or more of olive oil compared with rare or no consumption. In aggregate, these foods provide good sources of dietary fiber, something we all tend to need more of. The recommendation for about 30 grams of fiber per day, adjusted by age, gender, and body size, is derived from a meta-analysis of 185 studies that showed people who consumed a high fiber diet had 31 percent less heart disease and 16 percent less type 2 diabetes or colon cancer. Fiber is the polar opposite of ultra-processed foods—it slows digestion, lessens glucose spikes, and helps reduce cholesterol. What is referred to as the Western diet, rich in ultra-processed foods, is pro-inflammatory and linked with metabolic dysfunction, cardiovascular disease, diabetes, and colon cancer. A randomized trial of a high fiber, "microbiome enhanced" diet compared to the Western diet with the same percentage of calories (isocaloric) for macronutrients led to less gut absorption of over one hundred calories per day (with some people over four hundred calories per day). Another way to put it: pooping calories instead of absorbing them!

Compared with eating few or no nuts and seeds a day, eating just a handful is associated with a 20 percent lower risk of cardiovascular disease, as seen in more than forty studies. Nut consumption, despite high caloric density, has also been linked to lower risks of obesity and weight gain.

The whole package of all these goodies is known as the Mediterranean diet (see below). Happily, this diet has been assessed by multiple randomized trials, unusual in the field of nutrition science. Data from over 12.8 million participants in randomized trials and observational studies provide robust support for benefit of the Mediterranean diet for its association with a reduction of death from any cause—cardiovascular diseases, coronary heart disease, myocardial infarction, cancer, neurodegenerative diseases, and diabetes. That's a compelling list of outcomes!

| Mediterranean Diet | Goal |
|---|---|
| **RECOMMENDED** | |
| Olive oil | ≥4 tbsp/day |
| Tree nuts and peanuts | ≥3 servings/wk |
| Fresh fruits | ≥3 servings/day |

| Mediterranean Diet | Goal |
|---|---|
| Vegetables | ≥2 servings/day |
| Fish (especially fatty fish), seafood | ≥3 servings/wk |
| Legumes | ≥3 servings/wk |
| Sofrito | ≥2 servings/wk |
| White meat | Instead of red meat |
| Wine with meals (optionally, only for habitual drinkers) | ≤7 glasses/wk |
| **DISCOURAGED** | |
| Soda drink | <1 drink/day |
| Commercial bakery goods, sweets, and pastries | <2 servings/wk |
| Spread fats | <1 serving/day |
| Red and processed meats | <1 serving/day |

(Adapted from https://www.nejm.org/doi/10.1056/NEJMoa1800389?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200www.ncbi.nlm.nih.gov)

More than a decade ago, a trial of 7,477 participants, deemed at increased risk for heart disease, were randomly assigned to either a Mediterranean diet supplemented with extra-virgin olive oil or a Mediterranean diet with supplemented nuts, or a control diet of reduced dietary fat. A five-year follow-up showed a 30 percent lower risk of cardiovascular death, heart attack, or stroke with the two Mediterranean diets versus the control diet. The initial trial report had to be retracted and republished because of errors in random assignment and some protocol deviations, but with no substantive change in the results. That is one of the largest randomized trials in nutrition history, and it points toward the primary prevention benefit of the Mediterranean diet in people considered at high risk.

What about secondary prevention, people who already have manifested cardiovascular disease? Over thirty years ago, a trial known as the Lyon Heart Study randomly assigned about six hundred heart attack survivors to a Mediterranean diet or control and showed a 70 percent reduction of mortality during a five-year follow-up. In a more recent randomized trial conducted in people with established heart disease, the Mediterranean diet was compared with a low-fat diet in one thousand participants (83 percent were men) with follow-up of up to seven years. Cardiovascular events, such as heart attacks, strokes, or needs

for bypass surgery, were reduced by 26 percent with the Mediterranean diet, and among men by 33 percent. For more than twenty-five thousand women followed for five years, a higher adherence to a Mediterranean diet was associated with 23 percent reduction of all-cause mortality.

In older people, the impact of the Mediterranean diet on the gut microbiome has been assessed in a randomized trial of more than twelve hundred participants, aged sixty-five to seventy-nine years, compared with a habitual control diet. The Mediterranean diet promoted bacterial taxa associated with less frailty, reduced inflammation, and improved cognitive function. Diets of plant-based fiber and high in fermented foods (such as yogurt, cheese, and kombucha) have been shown to favorably modulate the gut microbiome, foster its diversity, and reduce immune-mediated inflammation.

Can we do better than the Mediterranean diet? A smaller randomized trial in 294 people with obesity, but no heart disease, tested three diets: Mediterranean, the green MED diet (adding walnuts, green tea, and a Mankai plant-based protein shake, with less meat and poultry), and healthy dietary guidance. The green MED diet showed some advantages over the Mediterranean diet by lowering low-density lipoprotein (LDL) cholesterol, inflammatory markers, blood pressure, and weight reduction. But this small trial only looked at blood markers and was insufficiently large to determine an effect on outcomes. While there may ultimately be a diet validated through randomized trials to be healthier, with better outcomes, than the Mediterranean diet, we haven't seen it to date.

Beyond an overall healthy food diet, there are some intriguing amino acids that deserve more attention than they have received. Taurine is not one of the nine essential amino acids (histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) we can obtain only from our diet. Much of the aggregate data supports that taurine decreases as we age (and in all species that have been assessed) and that its deficiency has been associated with diabetes, hypertension, liver disease, abdominal obesity, and inflammation. Everything about taurine has the look of it being a driver of antiaging, from suppressing mitochondrial dysfunction, to decreasing DNA damage and inflammation, to reducing cellular senescence. The impact of six months of taurine supplementation to aged rhesus monkeys, equivalent to humans ages

forty-five to fifty years, was notable for promoting health span: a 19 percent reduction in fasting glucose, increased bone density, and improvement of markers of mitochondrial function, liver function, and immune system. Taurine-rich foods include shellfish, especially scallops, clams, and mussels, and dark meat of turkey and chicken. Other good sources are brewer's yeast, milk, yogurt, cheese, and peanuts. It is not generally found in any plant foods, except red algae. Whether to take a taurine supplement to promote healthy aging has not been established and is thus far without verification from any randomized trials.

Choline is an organic compound, not an amino acid, that is considered an essential nutrient that must come from our diet. It is important for multiple brain functions. Rich sources are eggs (particularly the yolk), beef, milk, shitake mushrooms, soybeans, chicken breast, cauliflower, broccoli, and fish. The National Academy of Medicine has recommended 550 milligrams or 425 milligrams per day for men and women, respectively. Like the nine essential amino acids that we must obtain from our diet, ingestion of choline, at the level recommended, is usually not an issue on a diet of the healthy foods reviewed above. For people aged sixty-five and older, choline supplementation has been linked with improved cognitive performance, but, again, randomized trials will be necessary to resolve this question.

## Caloric Restriction

There has been considerable interest in whether calorie reduction or time-restricted eating leads to healthy aging. During World War I in Denmark, data from a two-year natural experiment showed dietary restriction associated with a 34 percent reduction in mortality. A standard approach entails a 20 to 50 percent reduction in calories, with preservation of macronutrient ratios. That differs from a ketogenic diet, which markedly reduces carbohydrate intake to less than 50 grams per day and derives 70 to 80 percent of calories from fat. Calorie restriction has been intensively studied for promoting longevity in multiple model organisms, such as yeast, fruit flies, and mice. In rhesus monkeys, however, one study reported marked increase in lifespan, and another showed no difference.

Less stringent, more palatable alternatives to calorie restriction are time-restricted eating and intermittent fasting. Time-restricted eating usually refers to a schedule of eating only for eight hours a day, with no caloric intake the other sixteen hours (known as 16:8). Intermittent fasting varies from alternative day fasting to a 5:2 schedule of fasting two days each week. Compared with regular food intake that is throughout the day and evening every day, these diets have been shown to match the benefits of more extreme calorie restriction, in particular the effects on insulin-signaling and other pathways. The participants in the many small, randomized trials vary considerably from being lean, being overweight or obese, or having type 2 diabetes. Some incorporate intensive coaching and monitoring, while others don't. Of course, the problem with comparing a standard calorie-restricted diet and a time-restricted eating regimen is that eating only in a circumscribed time window results in some caloric restriction.

Perhaps the most important message here is that our digestive processes seem to work best when fed on a regular routine. But I'll review a sample of the trials to give you a flavor of their results, some of which are contradictory. A randomized trial of 139 participants with obesity found little difference between calorie-restriction and time-restriction diets with regard to weight loss and metabolic markers after twelve months. Similarly, in a twelve-week trial of 116 participants who were overweight or obese, time-restricted eating had no benefit in weight loss or metabolic parameters (that included insulin, glucose, lipids, activity, energy expenditure, and fat mass) compared with the usual diet of eating throughout the day. However, another randomized trial for weight loss in ninety participants with obesity found a time restriction early in the day (7 a.m. to 3 p.m.) versus the usual diet (continuous eating) was superior for losing weight and fat mass, lowering diastolic blood pressure, and improving mood and sleep. Another randomized trial of time-restricted eating compared with continuous eating in seventy-five people with type 2 diabetes demonstrated weight loss and improved glucose regulation. In a trial of 209 people at risk for type 2 diabetes, twenty-hour fasting periods three nonconsecutive days per week was compared to basic calorie restriction or continuous eating and provided only modest improvement in glucose regulation. Many small trials of intermittent fasting, be it

alternate days or two days a week, have shown parallel benefits, as did a systematic analysis published in 2024.

General conclusions about these results remain elusive. The problem with a strict calorie-restricted diet is that it is hard to adhere to, has been associated with loss of bone density and muscle mass, and slowed brain function. Our brain is only 2 percent of our body mass but consumes 20 percent of our calorie intake. On the other hand, multiple studies support an improved immune response, and, perhaps interrelated, potential antitumor effects show up in experimental models. No calorie-restricted, time-restricted, or intermittent fasting diets have been proven to provide antiaging properties, but a small, randomized trial showed reduction of a biological age score that relied on some improvements of biomarkers (immune system, liver, glucose regulation) with multiple cycles of a fasting-mimicking diet. Until there's more definitive evidence, perhaps the best practical advice is to eat an early dinner, at least three to four hours before going to bed, and avoid any caloric intake before the next morning's breakfast.

Several scientists involved in that Lancet Discovery Science study signed on to this perspective: "This fad for fasting—and the perception that it is a clear path to weight-loss and 'wellness'—creates opportunities for charlatans."

There's a tie-in to all of this with our sleep-wake cycle—our circadian rhythm—and gut microbiome. More than half of our gut microbiome flora rhythmically fluctuates throughout the day-night cycle. Our circadian physiology has diverse and important effects on all our organ systems, which include nutrient absorption and gut hormone production. Our central clock, set by light, resides in the brain's suprachiasmatic nucleus, whereas our peripheral clocks are distributed throughout organs and tissues in the body. Disruption of timing of our meals desynchronizes the clocks and induces glucose intolerance. The immune cells of our gut, the gut barrier, and inflammatory response are affected by our clock system. That's why it's helpful to maintain the same pattern of consumption from day to day.

## Supplements

Despite their popularity and heavy marketing, there's little or no hard evidence for the benefit of taking any vitamins or supplements, especially in those who are following a healthy diet. Vitamin D has been advocated for prevention, but a wide variety of randomized trials, and large observational studies have failed to back its benefit. A large, randomized trial of omega-3 fatty acids or placebo in nearly twenty-six thousand participants with over five-year follow-up failed to show any benefit for preventing cardiovascular disease or cancer. A *possible* exception is that there may be a modest slowing of cognitive aging in older adults taking daily multivitamins, as seen in a randomized trial among 573 participants who took a Centrum Silver multivitamin or matching placebo daily (specifically improved episodic memory and global cognition). The authors added their results to previous randomized trials of about five thousand older adults and then claimed the benefit corresponded to "a two-year reduction in brain aging," which was met with objection by neuroscience experts. Indeed, randomized trials of multivitamins have shown no effect for preserving cognition, such as one in six thousand men tracked for twelve years. A comprehensive assessment of 277 randomized trials testing vitamins and supplements failed to show survival benefit or reduced cardiovascular mortality for vitamin A, B complex, C, E, selenium, antioxidants, calcium, fish oils, and many others widely used. Furthermore, evidence emerged that calcium plus vitamin D might increase the risk of stroke and cardiovascular mortality, as seen in a large, randomized trial in older women. Niacin intake appears to be associated with increased cardiovascular risk by promoting inflammation. There remain many candidates that have not been adequately tested, some referred to as "longevity vitamins," such as taurine and choline mentioned earlier and several others. For now, I say take them with a grain of salt.

## The AI Diet

The idea of a one-size-fits-all universal diet—one that is optimal for all people—is naive. Each of us is biologically and physiologically unique with respect to our

genome, metabolism, gut microbiome, and environment. Our food intake is, at least in part, determined by our genetic makeup, with specific genomic variants linked to food type preferences. There are hundreds of known gene variants that interact with our food intake, such as milk in preventing type 2 diabetes in people who have a lactose deficiency variant. While we've known about the potential merits of an individualized diet, it would require crunching billions of pieces of data for each person, such as analyzing the forty trillion bacteria from about one thousand species that reside in our gut, along with our medical conditions, diet, exercise, sleep, medications, and many other layers of data, like the three billion letters of our genome and over twenty-six thousand chemicals found in foods.

If you have pronounced spikes in your blood glucose after eating, you are at higher risk for cardiometabolic disease—even if you have a normal fasting sugar level. These spikes can be accurately measured with a continuous glucose wearable sensor placed on the arm or abdomen. In response to the exact content and amount of food intake at the same time ingested, there is marked variability from one person to another with respect to these glucose spikes. That makes glucose a window into an individual's nutritional health. An increasing number of healthy people are trying wearable continuous glucose monitor (CGM) sensors[I] that can be placed on the arm or belly and give accurate measurements for one to two weeks. That was reinforced with the FDA approval of CGMs for direct-to-consumer purchase, when until 2024 they had required a doctor's prescription. From CGM data, most people will discover they experience glucose spikes of varying magnitude in relation to what they eat and drink, how they exercise and sleep, and their stress levels. While such data help people learn their individual triggers for spikes, we do not yet have clear data that avoiding spikes means fewer cases of type 2 diabetes, metabolic syndrome, heart disease, or other health outcomes. Nevertheless, the idea of avoiding glucose spikes has set off a craze with influencers like Jessie Inchauspé, known as the Glucose Goddess, and many companies, such as Level and Signos, promoting CGM use.

It took a landmark 2015 paper from the scientists at the Weizmann Institute in Israel to lay the foundation for personalized nutrition. They studied eight hundred participants without diabetes in depth. This was an enormous dataset with about 1.5 million continuous glucose sensor measurements to more than

five thousand standardized meals, another forty-seven thousand meals of usual food intake, and data from the participants' gut microbiome, blood tests, and physical measurements. With a machine learning model that accounted for more than one hundred factors that could be involved in glucose spikes, the key determinant turned out to be the person's gut microbiome, which is shaped by our diet, genetics, social contacts, and medications.

The importance of the gut microbiome for predicting our response to food was later confirmed and extended by a group in the United Kingdom using a similar big data and machine learning model approach, which added identical twins as part of the participant group, and measuring not just glucose but also triglycerides. The latter requires frequent blood test assessments since there is no wearable sensor for this key metric. Notably, there was no relationship between the glucose and triglyceride response in participants, telling us that tracking glucose alone, although helpful, is not sufficient to understand personalized nutrition. Further, it's not just the genes, since there were marked differences among identical twins in nutrition response. While these studies highlighted the gut microbiome as a dominant contributor for our unique response to food, many other factors such as sleep patterns, physical activity, and sequence of meal ingestion play a role.

The concept of personalized nutrition was tested prospectively in a randomized trial of 225 participants with prediabetes, comparing an algorithm for predicting glucose-response diet (based on clinical and microbiome data) with a Mediterranean diet. Over six months, the algorithmically based diet led to significantly improved glucose regulation. That might help people at high risk to prevent ever manifesting type 2 diabetes, or for others already diagnosed to achieve remission. But such outcomes have yet to be assessed. In another randomized trial of a personalized nutrition program, there were reductions in blood triglycerides, body weight, waist circumference, and gut microbiome diversity.

The National Institutes of Health is investing $189 million to study ten thousand participants as part of the All of Us Research program. Called the Nutrition for Precision Health, the data being collected are similar to the Israel and British studies but also include dual-energy X-ray absorptiometry scans, grip

strength, and photos of all the participants' food via an automatic ingestion monitor attached to eyeglasses. About five hundred people will live at a clinic site for six weeks for close monitoring.

The work using AI to determine an optimal diet for individuals is still in its early stages. In 2019, I wrote on the potential merits of AI, conveying my own multilayered data collection and the predictive algorithm directing me to many foods that were not just unalluring but, for my preexisting condition of kidney stones, potentially dangerous. It's also challenging because it's not likely a one-off determination; your optimal diet will likely change with stress, weight loss, and significant changes in your physiology. Eventually we will get there, with all of a person's data integrated, but whether it will be adopted or change clinical outcomes, such as preventing heart attacks or diabetes, will take years to determine. Worsening health inequities across society might seem more urgent for the health of our species.

## EXERCISE

Turning to a factor that seems more within our control with a profound impact on health outcomes: physical activity. Nothing surpasses regular exercise for promotion of healthy aging! Exercise can be viewed as the single most effective medical intervention that we know. If you could design a drug that exerted diverse potent salutary health impacts across all our organ systems, it would be considered a miracle breakthrough. Regular exercise leads to favorable adaptations of the cardiovascular system, brain, pancreas, skeletal muscle, gastrointestinal tract (enhanced production of short-chain fatty acids, SCFA), liver, adipose tissue, gut microbiome, and peripheral blood vessels. The metabolic trifecta of three organ systems—pancreatic, skeletal muscle, and adipose tissue—leads to increased insulin sensitivity. Further, exercise protects against atherosclerosis and improves cardiovascular function through multiple pathways, including reducing lipids in the blood, increasing lipid oxidation by skeletal muscle, reducing bone marrow output of white blood cells that lessens body-wide and brain inflammation, improving our immune system responsiveness, and, at the subcellular level, enhancing mitochondrial function. A randomized

trial showed inflammation biomarkers were suppressed more with exercise than with a GLP-1 drug. A major NIH initiative to understand the effects of exercise, the Molecular Transducers of Physical Activity Consortium (MoTrPAC), has provided multiomic data, in the experimental model and in people, that validate protective effects of physical training across multiple organs, such as the immune system, and confirm sex-specific effects, particularly for adipose tissue biology. When I discussed this with Euan Ashley of Stanford University, one of the leaders of MoTrPAC, he interpreted the body of evidence based on a London bus conductor versus bus driver study from 1958, along with many recent reports, as "one minute of exercise bought you five minutes of extra life. And, actually it's a little more. If you did high intensity exercise, one minute would give you seven or eight minutes of extra life." He tells this to his patients when they come in and say they don't have enough time to exercise.

Compared with no exercise, briskly walking 450 minutes per week was associated with living 4.5 years longer. That evidence comes from a cohort of more than 650,000 individuals followed for over a decade.

The multitude of associated benefits are seen for all major organ systems. As well as reduction in cardiovascular mortality and of cancer mortality, we see a reduction in all-cause mortality. A systematic review of 196 studies with more than thirty million participants reported the link to a 31 percent reduction of all-cause mortality, with a "dose response" of more activity, more benefit. A similar magnitude of benefit was seen for increased physical activity improving sleep in more than four thousand participants studied for ten years.

What does moderate and vigorous physical activity mean? Brisk walking, bicycling on level ground, dancing, or gardening, accompanied by an increase in heart rate and breathing but you can still talk comfortably—that's moderate. Vigorous activity is breathing hard with a fast heart rate from jogging, swimming laps, competitive sports such as basketball or soccer, elliptical, stair climbing, and other aerobic activities.

A prospective study of nearly seventy-two thousand adults in the UK Biobank with a wrist-worn accelerometer and followed for about six years confirmed all these favorable outcomes, along with a linear dose-response relationship of increasing benefit with moderate to vigorous physical activity. A beneficial

relationship for reduced falls was seen in a large cohort of older women engaged in physical activity of more than 150 minutes per week and for preserving cognitive function in older adults. Overall, women benefit more than men from physical activity with respect to all-cause and heart-related mortality.

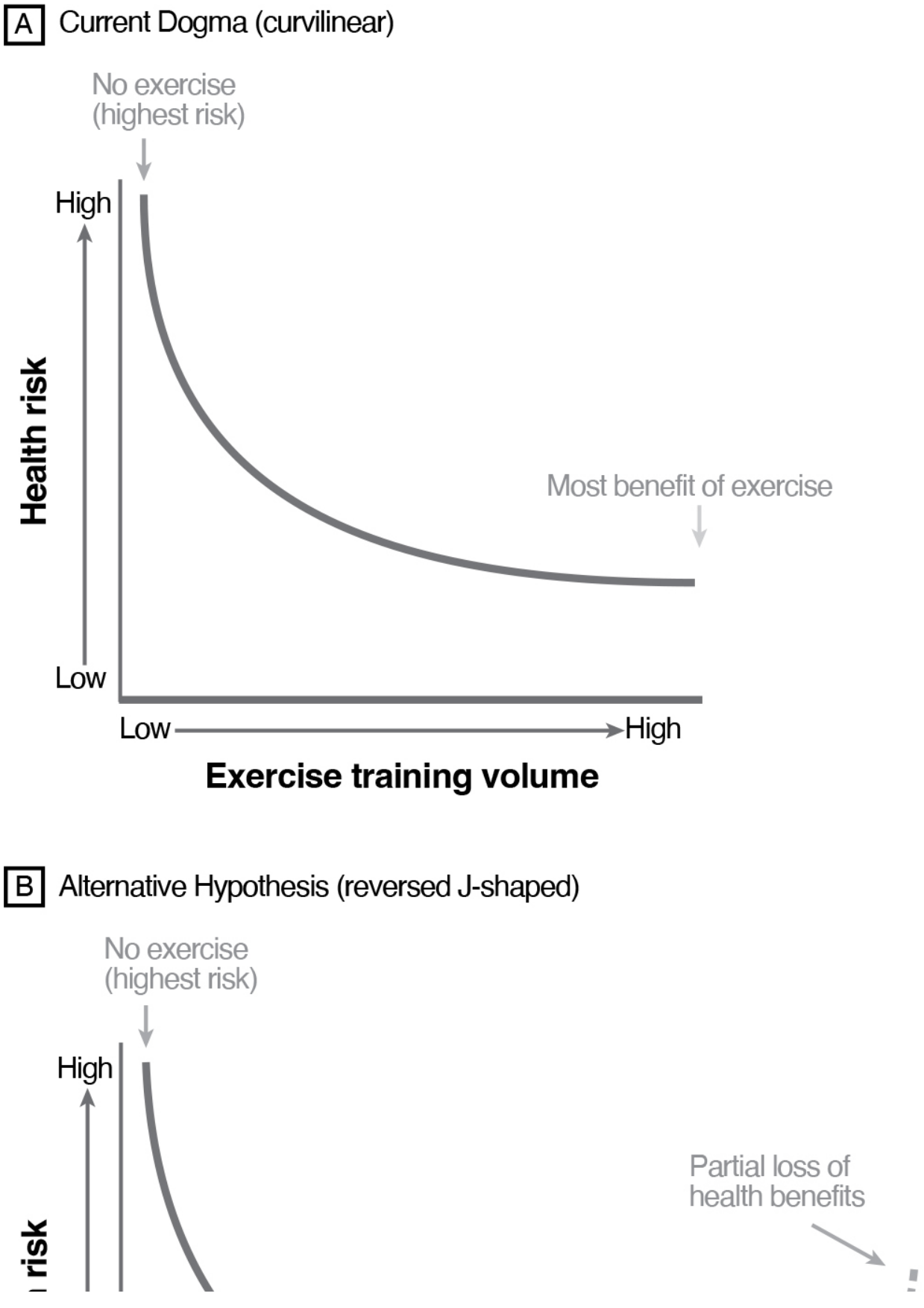
However, there is debate as to whether a plateau of benefit exists with moderate or vigorous exercise. On the one hand, a study of more than 400,000 Americans of mean age forty-three years followed for ten years showed no plateau of benefit for mortality reduction. On the other hand, a report on more than 116,000 adults with thirty years of follow-up linked reduction of mortality (all-cause, cardiovascular, noncardiovascular) in people who plateaued their physical activity at 300 to 600 minutes per week for moderate exercise or 150 to 300 minutes per week for vigorous exercise. A systematic review of many studies concurs with this finding, that higher doses are not necessarily associated with greater mortality benefits. Likewise, the large body of evidence from observational studies points away from the alternative hypothesis (fig. 3.5) of any loss of health benefits from too much physical activity.

Despite the wealth of evidence that supports improvement in longevity with exercise, only one in four American adults, by self-report, engage in adequate leisure-time aerobic and muscle strengthening physical activity that meets the guidelines of 150 minutes/week or more of moderate physical activity, or 75 minutes or greater of vigorous physical activity, *and* muscle strengthening activity at least two times per week. Globally, the proportion of adults with inadequate physical activity has been pegged at 27.5 percent.

Multiple studies have estimated the number of American adult deaths per year that could be prevented: with just ten minutes per day, 110,000 deaths; with thirty minutes, 270,000 deaths. Yet what is termed *fragmented* physical activity in older adults, of less than five-minute duration, or watching television for extended periods of time, has been associated with higher mortality risk.

What about steps? They are easily and, for the most part, accurately counted by wristbands, smartwatches, and digital accelerometer trackers. The frequently cited threshold of 10,000 steps per day has never been validated, whereas many studies have demonstrated benefit that starts at much lower levels, depending on the features of the cohort. For example, for a cohort of about 17,000 women with

a mean age of seventy-two years, there was an association of reduced all-cause mortality that was seen at 2,700 steps/day, and magnitude of benefit increased to a plateau at approximately 7,500 steps/day. A systematic analysis of twelve studies with over 110,000 individuals found the association of benefit starting at 2,500 steps per day (8 percent reduction of all-cause mortality) and progressive reduction up to 8,800 steps per day (60 percent reduction of all-cause mortality and cardiovascular events). There was additional benefit that noted how many steps were taken per minute, irrespective of the total step count. Coincidentally, the much ballyhooed 10,000-step goal that was advanced without empirical evidence was supported by a UK Biobank study of over 72,000 participants, which found the lowest mortality risk was seen for 9,000 to 10,500 steps per day. The value of walking was noted in an analysis of ten studies, with faster walking speeds, 2.5 to 5 miles/hour, offering more protection from developing type 2 diabetes.


A Current Dogma (curvilinear)
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(highest risk)
High
Health risk
Low
Most benefit of exercise
Low
High
Exercise training volume
B Alternative Hypothesis (reversed J-shaped)
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(highest risk)
High
Partial loss of
health benefits

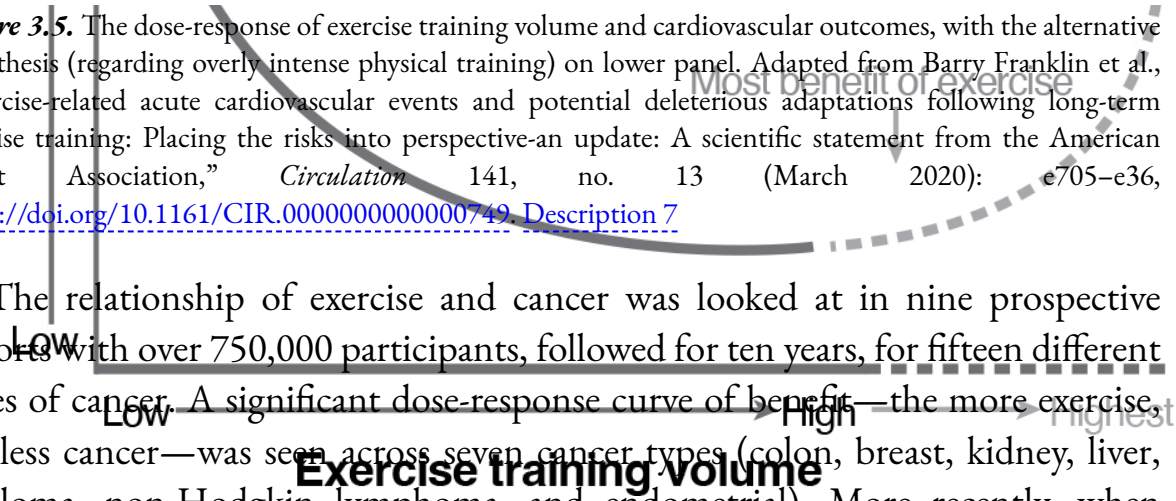



***Figure 3.5.*** The dose-response of exercise training volume and cardiovascular outcomes, with the alternative hypothesis (regarding overly intense physical training) on lower panel. Adapted from Barry Franklin et al., "Exercise-related acute cardiovascular events and potential deleterious adaptations following long-term exercise training: Placing the risks into perspective-an update: A scientific statement from the American Heart Association," *Circulation* 141, no. 13 (March 2020): e705–e36, https://doi.org/10.1161/CIR.0000000000000749. Description 7

The relationship of exercise and cancer was looked at in nine prospective cohorts with over 750,000 participants, followed for ten years, for fifteen different types of cancer. A significant dose-response curve of benefit—the more exercise, the less cancer—was seen across seven cancer types (colon, breast, kidney, liver, myeloma, non-Hodgkin lymphoma, and endometrial). More recently, when studied in more than 60,000 people for seventeen types of cancer, vigorous exercise was also associated with reduction of head and neck, lung, bladder, and pancreatic cancer.

More than 78,000 UK Biobank participants with wrist accelerometers were followed for seven years to determine the relationship between steps and all-cause dementia: a daily dose up to 9,800 steps was linked to protection, along with steps at high intensity or cadence. The relationship between physical exercise and cognition has been intensively studied, approaching two thousand reports, and eighty randomized trials. A review of the randomized trials suggests that the favorable impact of exercise is consistent but small. The mechanism has been tied to increased brain neurogenesis and plasticity, exercise's anti-inflammatory effect, and improved cardiovascular health.

The problem with using steps as a proxy for physical activity is that it doesn't represent discrete exercise time, a nonoccupational effort. The recommendation of 150 minutes per week of moderate physical activity is meant to consist of dedicated sessions, not incidental starting and stopping, with a sustained increase in heart rate, to promote aerobic cardiovascular fitness.

Over decades as a cardiologist, I would only stress the need for aerobic exercise, omitting the importance of strength, resistance, and balance training. That was a mistake, especially with all the evidence that has come out in recent years highlighting the loss of muscle mass as we age, and its prognostic importance. Aerobic conditioning should be considered just one of the two dimensions of exercise that are requisite. This activity could be push-ups, pull-ups, sit-ups,

squats, lunges, and using weights, bands, or machines, ideally getting at all muscle groups (arms, chest, abdomen, legs, hips, and back). A goal for building muscle power after taking the sit-to-stand test, which is how many times you can do that in thirty seconds (eleven to twelve for women and men over age sixty-five), can be achieved with squats, hinges, and pushes plus light weights, starting with five-pound dumbbells. Isometric exercises holding tension in the body, like wall squats for eight minutes a day, three times a week, lower blood pressure as effectively as aerobic exercise or high-intensity interval workouts, as seen from a review of 270 randomized trials. Unlike aerobic activity, the studies that have looked at the relationship between resistance training and all-cause mortality suggest sixty minutes per week may represent a plateau.

Muscle mass and strength start to go down at about fifty years of age. Between ages sixty and ninety years, men lose about 33 percent of their muscle mass and women about 26 percent. Muscle strength parallels that decline. Preserving core strength is essential for good posture and avoiding back pain and joint inflammation, all while promoting mobility and balance.

The associated reduction in all-cause mortality of about 25 percent (fig. 3.6) with sixty minutes per week of resistance training doesn't tell the whole story. Benefits extend to associations with less cardiovascular and cancer mortality, less visceral fat, better sleep quality, more bone density, enhanced balance, and improved mental well-being. A randomized trial of one year of resistance training in older adults compared to a nonexercizing control group found durable muscle strength benefit out to four years.

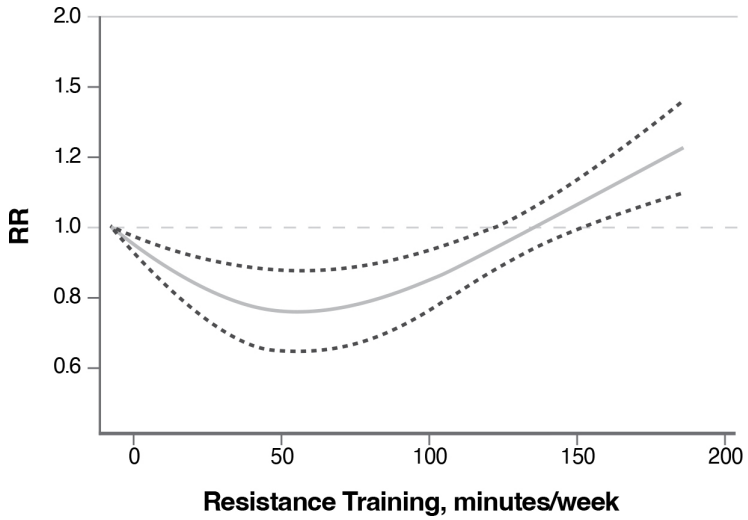



***Figure 3.6.*** The relationship of duration of resistance training and risk of all-cause mortality. Adapted from Prathiyankara Shailendra et al., "Resistance training and mortality risk: A systematic review and meta-analysis," *American Journal of Preventive Medicine* 63, no. 2 (August 2022): 277–85, https://doi.org/10.1016/j.amepre.2022.03.020. Description 8

Review of these data in aggregate prompted me to get into regular resistance and strength training, four to five times a week, for at least twenty to thirty minutes. If I'm going to be old, I'd rather be strong and old! I also opted to get a dual-energy X-ray absorptiometry scan, which can be obtained at relatively low cost (approximately $50–$75) to measure my bone density, muscle mass, and fat mass. My results are shown in figure 3.7. I don't understand why this test is not available or used in many medical centers, as it conveys so much useful information.

As discussed with protein intake related to preservation of muscle mass, grip strength, which is representative of overall strength, is an important prognostic metric. Unlike the curve for resistance training, the relationship between grip strength and all-cause mortality reduction is linear (fig. 3.8), as determined from forty-eight studies with over 1.1 million participants. Every 5 kilograms of hand grip makes a difference, and there are many ways your hand grip can be strengthened by squeezing stress balls or a tennis ball, or by using a pull bar at a gym, at least ten times a set, five to ten times a day. The three major types of grip strength are crush, as in twisting a cap off a bottle; support, as in holding onto an object like groceries; and pinch, holding a pen between fingertips and thumb. After the age of fifty, we lose an average of over 2 percent of grip strength per

year. A good move is to get a dynamometer—they are not expensive—test your grip strength, and work on getting it up to a higher level. The simple stress or tennis ball exercises should kick in within a couple of weeks. By itself, it may not have a material impact on your health span, since the association between grip and survival is not, by any means, established as cause and effect. But, at the least, it'll help with many practical tasks for years to come.

**Eric Topol** **Measured: 8/14/2024**

Age: 70.1 Gender: Male Birth Date: 6/26/1954 Height: 75.0 in.

| Regional Body Composition Analysis | | | | | |
|---|---|---|---|---|---|
| The regional body composition report below shows the 5 key regions of your body including your arms, legs, trunk, android (abdomen) and gynoid (hips region) metric and displays the composition analysis for each region. | | | | | |
| **Region** | **Total Fat %** | **Total Mass (lbs)** | **Fat Tissue (lbs)** | **Lean Tissue (lbs)** | **BMC (lbs)** |
| Arms | 19.3% | 20.2 lbs | 3.7 lbs | 15.5 lbs | 1.0 lbs |
| Legs | 21.8% | 59.2 lbs | 12.3 lbs | 44.3 lbs | 2.6 lbs |
| Trunk | 26.5% | 89.9 lbs | 23.3 lbs | 64.6 lbs | 2.0 lbs |
| Android | 27.6% | 13.6 lbs | 3.7 lbs | 9.7 lbs | 5.2 lbs |
| Gynoid | 20.3% | 29.2 lbs | 5.8 lbs | 22.7 lbs | 0.7 lbs |
| **Total** | **23.9%** | **180.7 lbs** | **41.6 lbs** | **132.1 lbs** | **7.0 lbs** |

# TOTAL BODY BONE DENSITY REPORT

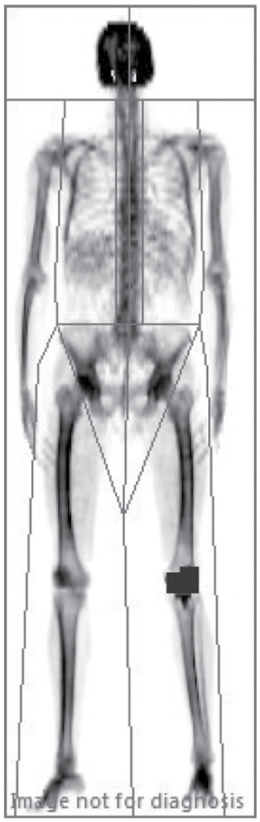
| Measure Date | BMD T-Score |
|---|---|
| 8/14/2024 | 0.0 |

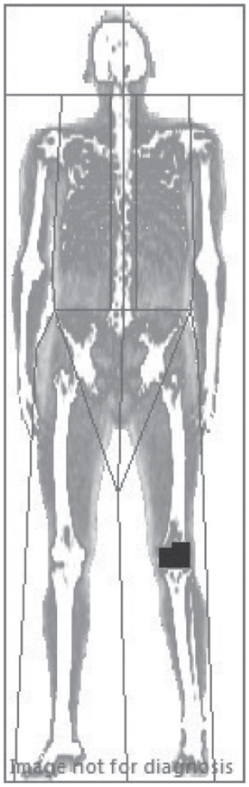
| Measure Date | BMD Z-Score |
|---|---|
| 8/14/2024 | 0.3 |

**T-Score: 0.0**

| | |
|---|---|
| -1 and above | Normal |
| -1.0 to 2.5 | Potential Osteopenia |
| -2.5 and below | Potential Osteoporosis |

| **Z Score: 0.3** | % Population (Greater Than) |
|---|---|
| **-1.5 to -0.5** | 7% - 30% |
| **-0.5 to 0.0** | 30% - 50% |
| **0.0 to 0.5** | 50% - 69% |
| **0.5 to 1.5** | 69% - 93% |
| **1.5 to 2.0** | 93% - 97% |
| **2.0 and above** | 97% - 99% |




Color Coding

Bone Lean Fat

***Figure 3.7.*** Results of my DEXA scan Description 9

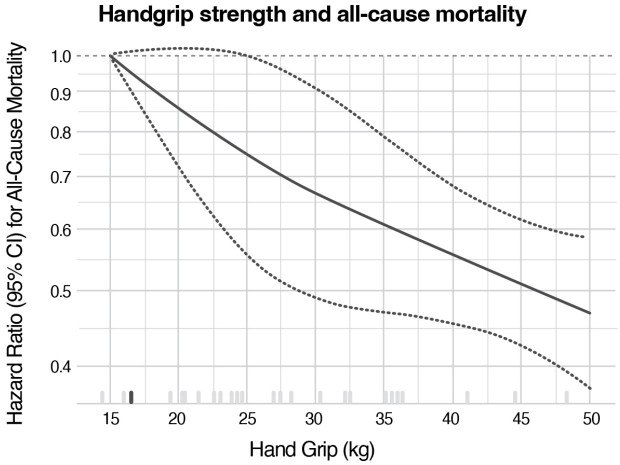



***Figure 3.8.*** The relationship between handgrip strength and all-cause mortality. Adapted from Ruben Lopez-Bueno et al., "Thresholds of handgrip strength for all-cause, cancer, and cardiovascular mortality: A systematic review with dose-response meta-analysis," *Ageing Research Reviews* 82 (December 2022): 101778, https://doi.org/10.1016/j.arr.2022.101778. Description 10

Before we leave types of exercise, we need to get into balance, best assessed by the one-leg stand test, adjusted for age. If you're sixty years and older, you should be able stand for thirty seconds or more. For a forty- or fifty-year-old, the goal would be at least forty-five seconds. As with grip strength and resistance training time, there is an association with all-cause mortality for people who cannot do a one-leg stand for ten seconds: it doubles. Balance can be improved by practicing one-leg stands or heel-toe walking. Stretching exercises, such as a forward bend, or with Pilates or yoga, helps flexibility.

There's one thing you don't want to do for any prolonged period: sit. While I was initially skeptical of the link between time sitting and risk of mortality, and the frequent notification alerts on smartwatches, many prospective studies have found consistent results, such as one with over 480,000 participants followed for thirteen years. People who sat mostly at work had an associated 16 percent higher all-cause mortality, and 34 percent increased cardiovascular mortality, after adjusting for potentially confounding factors. This report and others suggested that increasing physical activity can help mitigate the detrimental effect of sitting, but better to limit time of sitting and derive the benefit of physical activity.

We're now understanding the synergic interaction of exercise and diet better. In people with prediabetes achieving a 10 percent weight loss by calorie

restriction and multimodal exercise (movement + resistance), there was a much greater improvement in insulin sensitivity than by calorie restriction alone. The mechanism by which exercise protects against obesity, the metabolic syndrome, and type 2 diabetes is getting unraveled, with the discovery of a metabolite (N-lactoyl-phenylalanine, Lac-Phe) that is induced with exercise, and reduces food intake.

With respect to optimal timing of exercise, our clock proteins and circadian rhythm come into play, with some studies suggesting late afternoon or early evening is better than the morning for exercise performance while others find morning is preferred. Exercise in the fasted state may have some benefit for lipid utilization and glucose regulation. Overall, there is no clear perfect time, but good support exists for an individual to maintain the same pattern, be it early or later in the day.

Now for an inspirational message about exercise: it's never too late! Consider the story of Richard Morgan, a ninety-three-year-old man, who was reported in the *Journal of Applied Physiology*. In his seventies, he took up regular exercise for the first time in his life using a rowing machine in his backyard shed, with an average forty-minute workout each day. In parallel, he eats a high-protein diet and does two strength training sessions per week with dumbbells, lunges, and curls. He has now won four world championships for indoor rowing, and cumulatively rowed the equivalent of ten times round the globe. Extensive assessment at the University of Limerick in Ireland, where his granddaughter is a researcher in the physiology lab, showed his 165 pounds of body weight comprised of 80 percent muscle and 15 percent fat. His maximal rate of oxygen uptake, $VO_2$, is similar to those reported for young adults. Peak heart rate during exercise was 153 beats per minute, which would be closer to what we'd expect in a fifty-year-old. This remarkable case study tells us there's no age limit that precludes getting in shape to counter age effects, and even the potential for using exercise as a means of reversing aging.

## SLEEP

A restful good night's sleep can provide a magical sense of restoration and wellness. In recent years, we've gleaned a better understanding for what's going on while we sleep, and why it's so essential to our health. During sleep, metabolic waste products are transported out of the brain by cerebrospinal fluid and interstitial fluid. Not to be confused with our lymphatics and lymph nodes in the rest of our body, the brain's glymphatic system is a network of vessels that interacts with and complements the cerebrospinal fluid's capability to clear the waste products. During non-rapid eye movement sleep, there are large waves of cerebrospinal flow. Impaired waste clearance has been documented in young, healthy individuals with a single night of sleep deprivation, as reflected by increases in the beta-amyloid protein seen in brain imaging. Marked accumulation of this protein is considered a precursor to the development of Alzheimer's disease. The dynamics for cerebrospinal and interstitial fluid flow is influenced by neurovascular coupling and our circadian rhythm: during the day, our waste production is high and our vascular tone is increased, whereas slow wave neuronal activity is low. These dynamics are reversed during the night. In the words of Michael Grandner and Fabian-Xose Fernandez, "Sleep is a non-negotiable biological state required for the maintenance of human life... our needs for sleep parallel those for air, food, and water." The relationship between sleep and health outcomes is profound, including all-cause mortality, cardiovascular mortality, cancer mortality, risk of cancer, type 2 diabetes and metabolic dysfunction, immune system function, obesity, Alzheimer's disease, hypertension, stroke, female reproductive health, and mental and behavioral health. A brief review of a few relevant studies provides a sense of the big impact. From the UK Biobank cohort, nearly ninety-nine thousand participants with a mean age of sixty-two years using sleep trackers, sleep regularity index (SRI) (probability of an individual being in the same state of sleep or awake at any two time points twenty-four hours apart, averaged over seven days) was correlated with all-cause, cardiovascular, and cancer mortality (fig. 3.9).

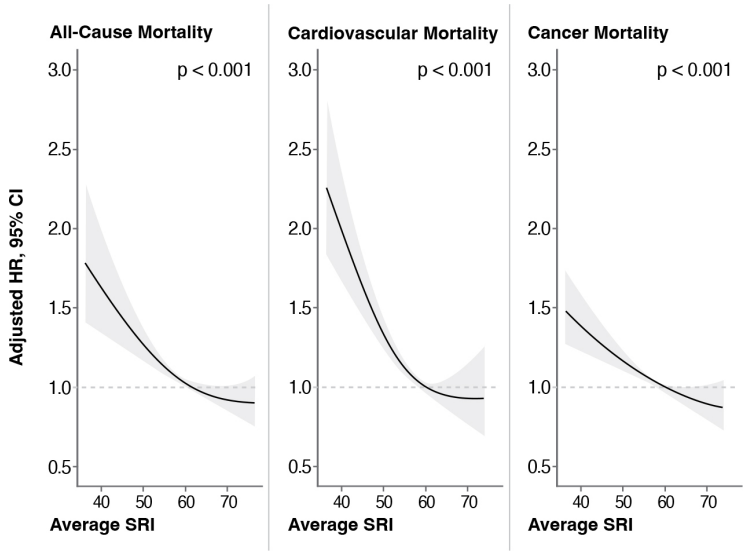



***Figure 3.9.*** The sleep regularity index (SRI) relationship (adjusted hazard ratio (HR), risk metric) for all-cause (A), cardiovascular disease (CVD) (B), and cancer (C) mortality. Adapted from Lachlan Cribb et al., "Sleep regularity and mortality: A prospective analysis in the UK Biobank," *eLife* 12 (November 2023): RP88359, https://doi.org/10.7554/eLife.88359. Description 11

Among nearly eight thousand participants with twenty-five-year follow-up, a sleep duration at age fifty to sixty for six hours or less, compared with seven hours or more of sleep, was associated with a 30 percent increased risk of dementia. In a randomized trial of people with habitual sleep duration of less than 6.5 hours and a BMI between 25 and 30 kg/m$^2$, simply coaching for better sleep led to an extra 1.2 hours of sleep and a significant decrease in daily caloric intake (270 calories). A study in young, healthy men who underwent adipose tissue and muscle biopsies after one night of deprived, as compared to full, sleep showed deleterious pro-inflammatory changes of the transcriptome, proteome, epigenome, and metabolites in the blood. The loss of one hour of sleep from daylight savings time in Germany and the United States was associated with a significant rise in heart attacks in both countries for four days.

So how much sleep do we need? One of the best studies to tackle this question used data from the UK Biobank, from nearly 500,000 participants, 48,000 of whom underwent brain imaging, and 156,000 had a six- to ten-year follow-up. The principal finding was that about seven hours is the optimal duration of sleep.

As you can see in figure 3.10, the relationship of sleep duration to health was not linear. It may come as a surprise that beyond seven hours there were

consistent signs, both acutely and during follow-up, of cognitive and mental health decline, as well as unfavorable changes in brain structure. Also consistent is the relationship of long sleep (more than eight hours) and heightened all-cause mortality (by approximately 30%) that was previously established among nearly 1.4 million people. To sum up all the studies of sleep duration and cardiovascular disease, every one hour a night decrease in sleep below the seven- to eight-hour threshold is associated with 6 percent higher risk of total cardiovascular disease. Every one hour a night increase in sleep duration above that seven- to eight-hour threshold is associated with 12 percent higher risk of total cardiovascular disease.

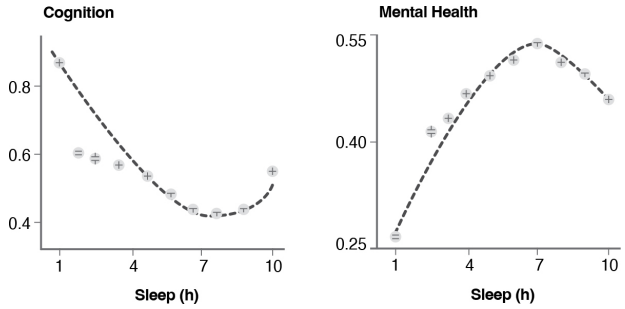



***Figure 3.10.*** Relationship of sleep duration with cognition and mental health from the UK Biobank study. Adapted from Yuzhu Li et al., "The brain structure and genetic mechanisms underlying the nonlinear association between sleep duration, cognition and mental health," *Nature Aging* 2, no. 5 (May 2022): 425–37, https://doi.org/10.1038/s43587-022-00210-2. Description 12

In 2017, Matthew Walker, a professor of neuroscience at UC Berkeley, sounded the alarm about the silent sleep loss epidemic as "the greatest public health challenge we face in the twenty-first century." Even before the COVID-19 pandemic, 35 to 40 percent of Americans were sleeping less than seven hours per night on a regular basis. Intercountry differences for sleep duration were addressed by a study of 1.1 million people with an aggregate of thirty-four studies of participants using sleep trackers in the Netherlands, the UK, and the United States. Americans got the least sleep. That same study zoomed in on insomnia symptoms by age. In people over sixty-five years of age, 15 percent had difficulty in initiating sleep, 20 percent had difficulty in maintaining sleep, and 34 percent had early morning awakenings.

The problem in older adults with sleep is threefold. There's less deep sleep, the precious non-rapid eye movement sleep substage that promotes health across all

systems, along with memory and cognition. By late age forties, it's already lowered more than 60 percent than in a teenager; by age seventy, it's down 80 to 90 percent. There's also more fragmented, disrupted sleep—reduced sleep efficiency—the percent of time asleep while in bed. On top of these disruptions, there is regression of circadian timing that leads to earlier bedtimes and earlier awakenings.

The master pacemaker clock for our circadian rhythm is in the suprachiasmatic nucleus, a small region within the hypothalamus, entrained by external cues from light. Exquisite molecular circuitry connects it with our muscle, liver, adrenal gland, kidneys, immune system, heart, and pancreas. Exemplifying the pivotal role of the twenty-four-hour sleep-wake cycle, there's a rare familial condition of short sleep with no adverse consequences due to a mutation in a molecular clock regulator gene. If only we could simulate that clock control, we might be able to promote better quality and efficiency of sleep with less duration (think genome editing someday in the future).

So we're faced with the challenge of promoting better sleep that is essential for healthy aging, while aging itself is compromising sleep quality. There are some things that help, like maintaining the same sleep pattern every day, including weekends. That includes a regular pattern of exercise and meals, with an adequate separation of multiple hours from bedtime (think early time-restricted eating). A cool and fully dark, quiet bedroom as well as avoidance of the blue light from electronic devices that disrupts circadian rhythm and suppresses melatonin production are simple tips. The benefit or risk of napping during the day has been a source of debate, but a large observational study found that napping one to two times weekly was linked to a significant reduction of cardiovascular events. Most epidemiologic studies that have looked at napping suggest their duration is important, and longer afternoon naps, especially more than one hour, are associated with risk.

In recent years, the popularity of sleep trackers has soared, including wearables such as the Oura ring, smartwatches, and fitness bands, and smartphone app "nearables" that can be on or beside the bed. Most of these devices rely on an accelerometer to detect movement during sleep and try to extrapolate the duration of REM, non-rapid eye movement sleep phases, and total sleep

duration. You can easily fake out that metric by reading in bed, which I have personally tested. More sophisticated devices, like Oura, integrate heart rate, movement, body temperature, and blood oxygen levels, which provides better alignment with formal sleep lab measurements, yet with the advantage of being in one's own bed. The problem is that there are no standards for the algorithms for their sleep scores and a general lack of validation for accuracy of reporting. Ironically, use of the trackers can induce or exacerbate anxiety, which interferes with sleep quality. On the other hand, self-diagnosis for triggers of poor sleep, such as alcohol, late meals, caffeine, or blue light, can be facilitated with their use.

Few rigorously assessed interventions have been shown to improve sleep quality. Professional organizations recommend cognitive behavioral therapy as first-line treatment because of the body of evidence that has borne out its efficacy. That treatment typically requires a trained therapist along with a significant time commitment and expense. However, in-person coaching of relaxation training, avoidance of stimuli, and improvement in sleep hygiene can be simulated with smartphone apps. A randomized trial of digital cognitive behavioral therapy, using a smartphone app, enrolled over seventeen hundred participants with insomnia and showed improvements of insomnia symptoms along with functional health and psychological well-being. This report and others are encouraging nonpharmacologic ways of improving a healthy sleep schedule.

Supplements such as melatonin or magnesium have been studied in many small randomized trials and have been shown to have relatively small effects for promoting sleep quality, and the quality of evidence for both was deemed low. The same problem with low-quality data exists for the supplement ashwagandha and the prescription medicine trazadone. Several companies are heavily promoting beds that regulate body temperature to promote sleep quality, but again, evidence that they work is thin at best. Many ongoing drug discovery programs aim to find an effective and safe medication to fulfill the unmet need.

Before leaving sleep, I would be remiss not to highlight sleep apnea, a common disruption of sleep that affects 34 percent of men and 17 percent of women in the United States. The most common symptom is excessive daytime sleepiness, but even that is not reported in more than half of people affected. Other symptoms include loud snoring, episodes during sleep when you stop breathing as reported

by another person, gasping for air during sleep, and difficulty staying asleep. When suspected, the diagnosis can be made with a home sleep apnea testing with about 80 percent sensitivity and specificity, preempting a formal sleep lab study. In my experience, avoidance of such formal studies is desirable. They are nonphysiologic and expensive, and it is rare to see people get anything but abnormal results. For individuals who are symptomatic, multiple interventions ranging from weight loss and exercise to oral appliances that hold the jaw forward, to positive airway pressure devices, to surgery, have been shown to be effective. Since sleep apnea is associated with a twofold or greater risk of cardiovascular and metabolic diseases, its diagnosis and management in symptomatic individuals is of obvious importance.

## ENVIRONMENTAL TOXINS

The health dangers of air pollution and other environmental toxins have recently become far more obvious, too. The Institute for Health Metrics concluded that "particulate matter air pollution was the leading contributor to the global disease burden." Even short-term exposure to inhalable particulate matter (PM) of 10 μm or less ($PM_{10}$) and fine (less than 2.5 μm) particulate matter ($PM_{2.5}$) is associated with increased all-cause, cardiovascular, and respiratory mortality, with each PM type exhibiting independent association with these outcomes.. About one million deaths globally per year are attributable to short-term fine particulate matter exposure. The supralinear relationship between fine particulate air pollution and mortality means there is a steep slope of increased risk even at low exposures. No safe threshold has been identified for chronic effect of $PM_{2.5}$ on cardiovascular health. An estimated 20 percent of type 2 diabetes is related to chronic exposure of fine particulate matter (fig. 3.11). The risks for $PM_{2.5}$, nitrogen dioxide, and ozone have been documented to exist at levels that are below current national air quality standards. Cognitive decline, a reduced immune response to COVID-19 vaccination, and increased blood pressure from rush-hour traffic have also been associated with air pollution. The mechanisms for these multiorgan system harms include body-wide inflammation, compromised immune function, increased oxidative stress, autonomic nervous

system dysfunction, and pro-clotting effects (fig. 3.11). Rather than air pollution's $PM_{2.5}$ induction of genomic mutations being the primary driver of lung cancer air pollution, activating immune cells and the inflammation they trigger appears to play a pivotal role for increasing the risk of lung cancer. Outdoor pollution, with PMs and ozone, was estimated to account for about 6 percent of global mortality, now over 8 million annual premature deaths, more than 80 percent of which could be addressed by phasing out fossil fuels. About 130 million Americans live in places with failing grades for unhealthy levels of ozone or particulate matter pollution, and that number has been increasing rapidly. The climate crisis, with new peaks in land and ocean surface temperatures and widespread wildfires, with synergistic hazard of extreme heat and wildfire smoke, is unquestionably making matters worse.

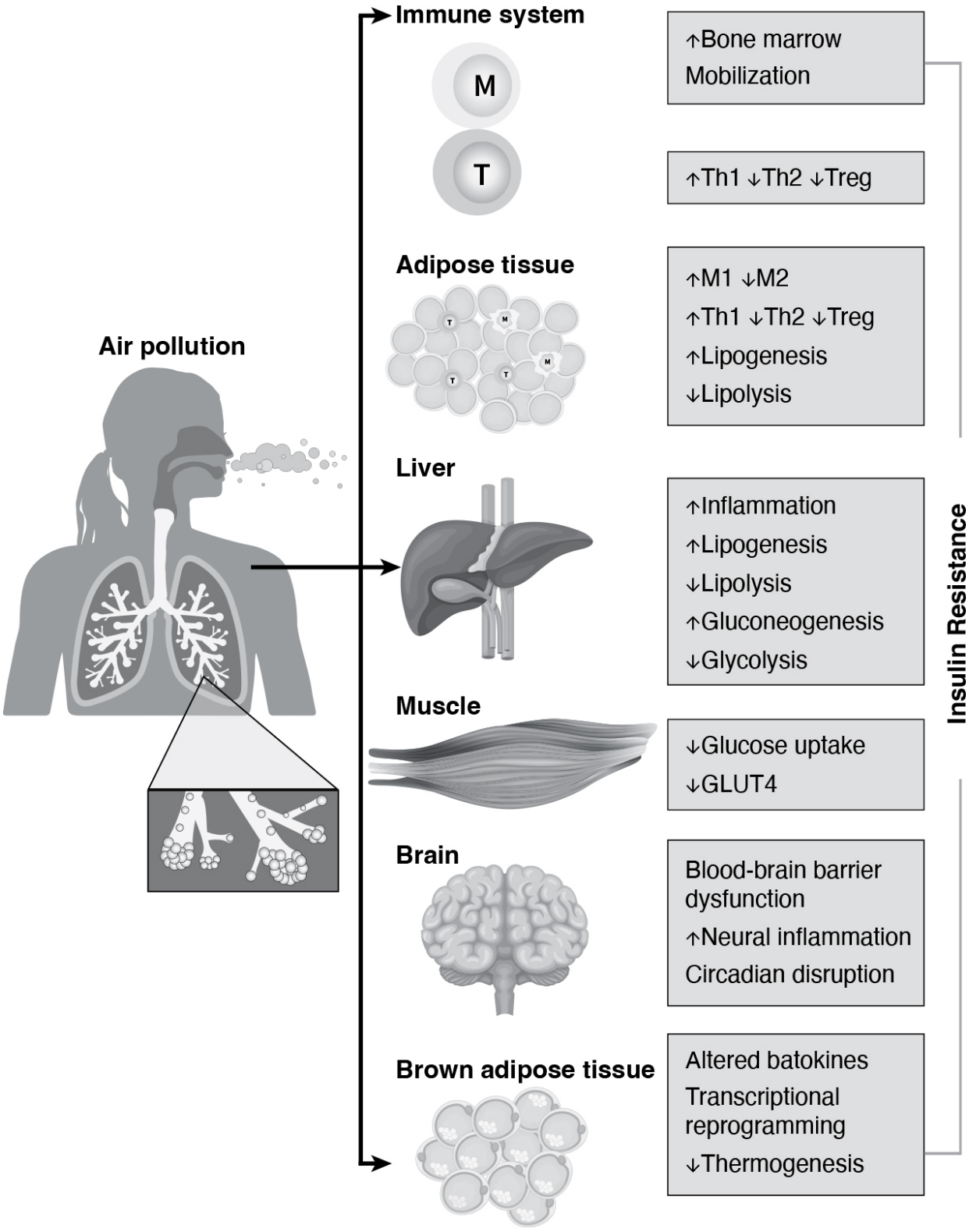



***Figure 3.11.*** The multisystem toxicity of air pollution. Adapted from Sanjay Rajagopalan et al., "Air pollution exposure and cardiometabolic risk," *Lancet Diabetes & Endocrinology* 12, no. 3 (March 2024): 196–208, https://doi.org/10.1016/S2213-8587(23)00361-3. Description 13

It's not just air pollution in our environment that poses significant risks. Secondhand smoke has been linked to ischemic heart disease, stroke, type 2 diabetes, asthma, and breast cancer. Moderate levels of the indoor odorless gas radon are associated with increased risk of nonsmoking lung cancer and risk of stroke, but 75 percent of American homes have not been tested for radon. About one-third of the population is currently exposed above a significant threshold level, which is fourfold higher than previous estimates.

Pesticides have been associated with a risk of multiple cancers, type 2 diabetes, cognitive impairment, Parkinson's disease, autoimmune diseases, thyroid disease, infertility, and loss of smell. A systematic review of the published studies found supportive but not definitive evidence for some of these risks, owing to rigorous, prospective assessment. *Consumer Reports* published practical recommendations for how pesticide exposure can be reduced from our foods based on several years of data from the Department of Agriculture. The pesticide health risks could be reduced by banning the implicated organophosphates and carbamates, but no action has been taken.

Besides gases, there are the health risks of noise pollution well beyond damage to our ears. About one hundred million Americans experience a continuous daily exposure of greater than seventy decibels, the equivalent of standing next to a washing machine all day. Chronic noise pollution, especially at nighttime, is associated with increased stress hormones, body-wide inflammation, high blood pressure, heart disease, and type 2 diabetes.

Microplastic (1 μm to 5 mm) and nanoplastics (less than 1 μm, smaller than a speck of dust) carry over ten thousand chemicals such as bisphenols and phthalates; have become ubiquitous in our environment, food, ambient air, the oceans, and drinking bottled water; and are linked with serious health hazards. The increasing plastic production each year, from less than two million tons in 1950 to more than four hundred million tons today, has led cumulatively to over six hundred billion tons, which will never go away. There are over 240,000 particles in an average liter of bottled water; 90 percent are nanoplastics. It was one thing to document the pervasive presence of microplastics in our air and water, but another to find it in our arteries, brains, blood clots, liver, gut, lung, placentas, testicles, and other tissues.

In an important study of more than three hundred patients who underwent carotid artery surgery and had their atherosclerotic plaque analyzed for microplastics, the majority had evidence of polyvinyl chloride or polyethylene in their arteries. Not only was the presence of the microplastic correlated with marked inflammation of the arteries compared with the patients not harboring plastics but there was more than fourfold heightened risk of heart attack, stroke, or death during three years of follow-up. In a study of microplastics in the testes, all twenty-three men had these detected, at a level threefold compared with canine testes, and their presence was associated with less weight of the testes and lower sperm count. In people with the autoimmune condition of inflammatory bowel disease, fecal microplastics were significantly elevated compared with healthy controls, and their level correlated with the disease severity. In the blood clots from patients with a heart attack, stroke, or deep vein thrombosis, microplastics were detected in 80 percent. Microplastics have been detected within human brain cells, including patients with brain tumors. In response to exposure to microplastics, cancer cells spread at an accelerated rate and transfer the particles to daughter cells when they divide. Association with increased risk of asthma, various cancers, interstitial lung disease, cognitive decline, cardiometabolic disease, neurodevelopmental delay, premature births, and infertility have been described.

In 2024, *Consumer Reports*' cover article "How to Eat Less Plastic" ranked eighty-five specific food products (Annie's *organic* cheesy ravioli came out on top with 53,579 ng total phthalates per serving, followed by Wendy's crispy chicken nuggets at 33,980 ng). Strategies suggested to limit exposure included avoiding plastic food storage containers, fast foods, and high-fat foods (higher level of plasticizers that are fat soluble); using water bottles made of glass or steel and kitchen tools made of wood or steel; and limiting use of vinyl. Despite the urgent need to address the ever-growing plastic crisis, little has been done.

Like forever plastics, the man-made perfluorinated or polyfluorinated alkyl substances (PFAS) have such strong chemical fluoride to carbon bonds (F to C, the key feature of *forever chemicals*) that they don't break down. Of more than forty-five thousand water samples the Environmental Protection Agency tested for PFAS, 31 percent were considered harmful and were not located near any

incriminating source for contamination. High exposure to these compounds has been linked to kidney and testicular cancer, obesity, increased blood pressure and preeclampsia during pregnancy, high cholesterol, inflammatory bowel disease, and thyroid, liver, brain, and immune system damage. Global studies indicate that the United States and Australia have the highest concentration of PFAS in their water. Just about all Americans have measurable PFAS in their blood.

Addressing the burden of PFAS in tap water could reduce exposure by 20 percent in the United States. To name just some of the other plethora of sources of PFAS: foods such as butter, processed meat and seafood, clothing, carpeting and furniture, dental floss, fast-food wrappers, microwave popcorn bags, pizza boxes, yoga pants, sneakers, solar panels, nonstick cookware, nail polish, firefighting foam, and artificial turf. And here's a double whammy: most plastic sandwich bags have high levels of toxic PFAS. The 3M company still uses these in sixteen thousand of their products, and their multidecade cover-up was recently exposed after an employee's discovery of PFOS (perfluorooctanesulfonic acid, a PFAS) in the blood of people who *didn't* work at the company.

Of more than ten thousand PFAS chemicals, the Environmental Protection Agency announced in 2024 its first ever drinking water PFAS standard to regulate, limiting the amounts of only six of them. Clearly, as with microplastics, we have a long way to go.

Ionizing radiation medical imaging studies, including CT (computed tomography, also called CAT) scans and nuclear imaging, are performed far too frequently in the United States, and have been associated with an increased risk of cancer.

There's also the concern about exposure to metals, including cadmium, tungsten, uranium, cobalt, copper, and zinc, and their relationship to cardiovascular disease. A prospective study of more than sixty-five thousand ethnically diverse participants assessing urinary levels of these six metals found a marked increase in cardiovascular events and all-cause mortality, proportional to the elevated levels.

All these chemicals and pollutants add to what I've reviewed on ultra-processed foods, contributing to health hazards that are not adequately

recognized or being handled. For example, the marked rise of various cancers in younger adults in recent years may be attributable to these environmental factors.

## SOCIAL ISOLATION AND THE BIG PICTURE

The attention to loneliness and social isolation as a critical public health concern has been mounting. Ninety cohort studies that were systematically reviewed, which included over 2.2 million people, demonstrated an association between loneliness and a 32 percent increased all-cause mortality, a 34 percent rise in cardiovascular mortality, and a 24 percent higher cancer-related mortality. It's important to note that the cause-and-effect relationship of loneliness and these adverse outcomes has not been established. Living alone, without having family and friends visit, without weekly groups, contributes to social isolation. People with obesity are far more likely to face social isolation and loneliness than those without obesity. A UK Biobank study that included nearly four hundred thousand participants with a mean age of fifty-six years, and thirteen years of follow-up, found that among the 23 percent of people with obesity, the less loneliness and social isolation, the lower all-cause mortality. We're in the earliest stages of determining whether generative AI tools can help reduce loneliness, supplementing or substituting for interactions with people, but a study using GPT-3 in a student population with loneliness and suicidal ideation provided some encouraging preliminary results. We may not like the idea of machines helping our kids avoid suicide, but if such AI works, can we really choose to not use it at least sometimes? Assessing lifestyle+ factors should include the extent to which you or those you care for exhibit social isolation and loneliness, and all the possible countermeasures.

Virtually everything discussed in this chapter has a connection with social determinants of health. A pivotal paper in 2017 on determinants of premature mortality among 1.7 million people provided unequivocal support for the importance of socioeconomic status, an independent risk factor. Socioeconomic status determines one's likelihood of good health as much as cigarette smoking, high alcohol intake, hypertension, type 2 diabetes, or physical inactivity. More unprocessed food in diet, poor sleep, more air pollution, and less physical activity

are disproportionally found in people with lower socioeconomic status. The first systematic review on the association of education and adult mortality showed a dose-response with a reduction of mortality risk of about 2 percent for every additional year of education. If we are to see a population-wide improvement in health span, the marked inequities must be reduced. Food deserts are real. More than sixty-five hundred of them are in the United States. Places where it is difficult to get healthy food, populated by a total of twenty-four million people. One study used GPS tracking for ninety-four million visits to 359,000 food retailers over two years. Most people travel beyond their neighborhood to buy food, an average of 3.7 miles. Travel to retailers that sold healthy food had a stronger association with the prevalence of obesity and cardiometabolic conditions than the percentage of retailers in a local area. Food insecurity, affecting more than fifty million Americans, has been linked to all-cause premature deaths in a study of over fifty-seven thousand adults, with about a 50 percent increase compared with people with full food security. Food-as-medicine programs to address food deserts and food insecurity have attracted considerable attention, but randomized trials have not yet borne out improvement in clinical outcomes. So much more dedicated efforts are required to combat inequities so that improvements in health span that are occurring will not be restricted to people of high socioeconomic status.

My expanded view of lifestyle makes a measurable difference to human health span. According to a modeling study for Americans, a sustained change in a typical Western to an optimal diet as we've reviewed, starting at age twenty years, would increase lifespan by more than ten years. Similarly, a UK Biobank modeling study for people aged forty calculated that adopting a healthy diet led to a nine-year increase in life expectancy. Among fourteen thousand Americans, those who didn't smoke, were not obese, and drank alcohol in moderation lived an average of seven years longer than their peers without this trio of lifestyle factors.

Another study, of more than seven hundred thousand US veterans, showed there was even more to gain in life expectancy. The cumulative effect of all eight factors tallied in these vets—never smoking, physical activity, no excessive alcohol consumption, restorative sleep, nutrition, stress management, social connections,

and no opioid disorder—was associated with twenty-four years of added life expectancy at age forty for males and 20.5 years for females. A lifestyle score based on those same factors was associated with increased likelihood of becoming a centenarian. A prospective study of healthy lifestyle, consisting of never smoking, BMI less than 25 kg/m2, high-quality diet score, regular exercise, and no more than moderate alcohol intake, was associated with marked improvement in healthy aging, of about eight to ten years without cancer, cardiovascular disease, or type 2 diabetes, as seen in figure 3.12 for people at age fifty. A favorable lifestyle was linked to lower risk of dementia across the range of low to high genetic risk. The capacity for lifestyle to mitigate genetic predisposition, calculated by polygenic risk score, was highlighted in a UK Biobank study with a thirteen-year follow-up that showed its favorable impact for over forty diseases.

This has been a survey of the lifestyle factors that are critical to healthy aging. The broad, body-wide, marked impact of these factors is also a function of their interdependence. It's not just diet, just exercise, just sleep, or just social isolation. Our environmental exposures are far more important than have been generally acknowledged. All of them come together to create a healthy lifestyle+ package. They may be considered "low tech" since much has been known about some, particularly diet and exercise, for thousands of years, but they have been refined and our knowledge has grown tremendously. My overall sense is that it will be hard for any new drug or intervention to match overwhelming consequences of a well-guided healthy lifestyle, but that doesn't mean it isn't worth reaping the advantages of the combination.

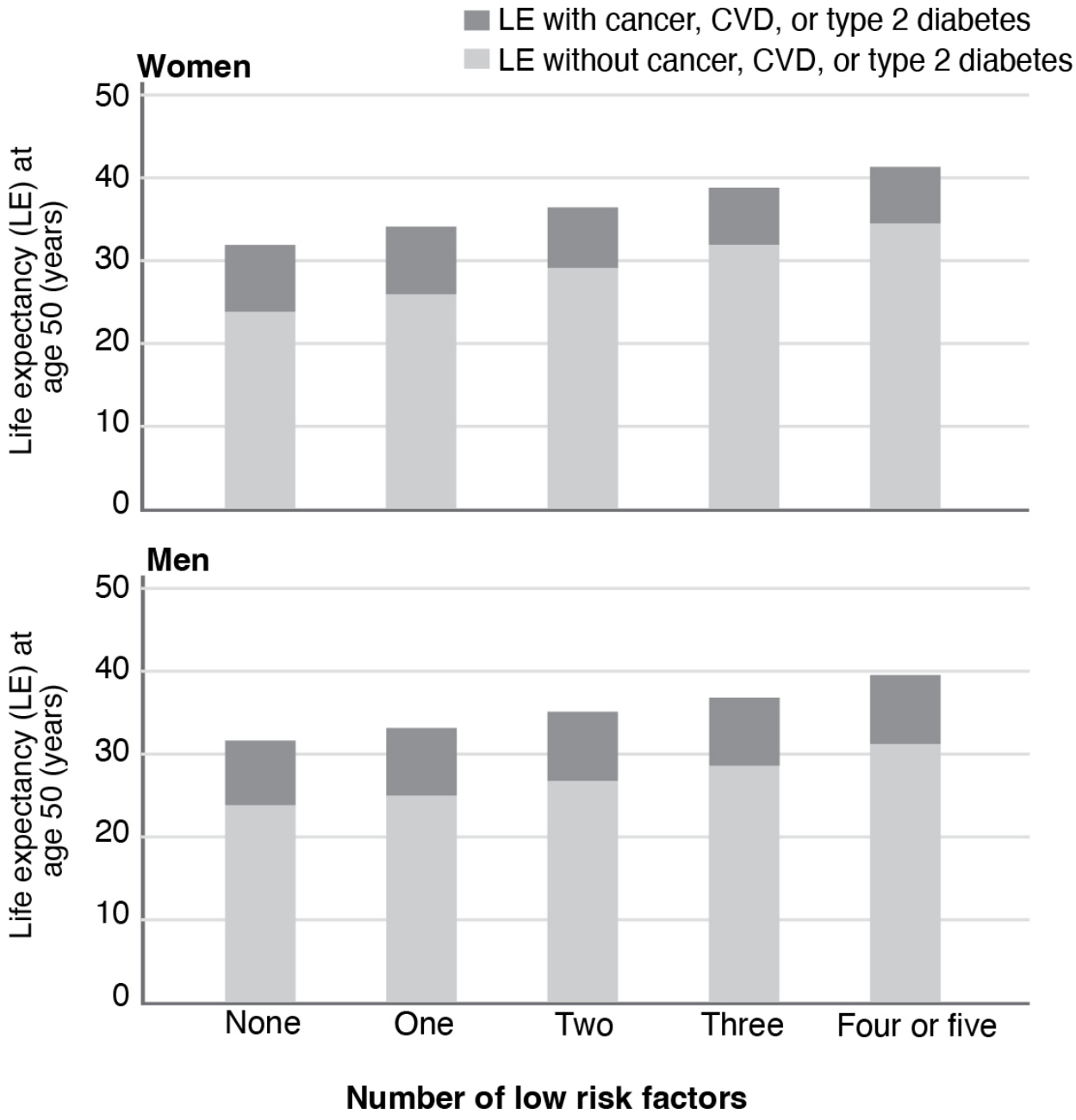



***Figure 3.12.*** Relationship of life expectancy (LE) and number of lifestyle risk factors (never smoking, no obesity, diet quality score, physical activity, and level of alcohol intake). Adapted from Yanping Li et al., "Healthy lifestyle and life expectancy free of cancer, cardiovascular disease, and type 2 diabetes: Prospective cohort study," *British Medical Journal* 368 (January 2020): 16669, https://doi.org/10.1136/bmj.16669. Description 14

We're ready to go high tech and see why rebooting our approach to dreaded diseases, and diagnosing them earlier, make them eminently more treatable. And even better, with the medical forecasting of high risk of a specific condition in an

individual, we can prevent it from occurring. Once determining such risks, our ability to prevent or substantially delay age-related diseases will always substantially depend on attention to lifestyle+ factors.

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I. For disclosure, I previously served on the board of directors at Dexcom, which makes continuous glucose monitors.

# PART II

Chronic Killers

# 4

# Obesity and Diabetes

One of the most life-changing medicines in history—based on the discovery of glucagon-like peptides or GLP-1s—sat under our noses for over twenty years before it began making a transformative difference to human health span. Glucagon and GLP-1s are naturally occurring hormones that arise from our gut and serve a critical role for managing the body's metabolism. The new GLP-1 medications mimic the structure of the natural hormones and can achieve far greater levels of these hormones in the blood. The first GLP-1 drug, exenatide, was approved in 2005. It took nearly a decade before a GLP-1 drug was available as a once-weekly injection instead of twice a day. Now these longer-acting drugs, such as Ozempic, Wegovy, Mounjaro, and Zepbound, are the rage and cannot be manufactured fast enough to keep up with the demand. The discovery of a venom in the saliva of the Gila monster, containing a long-acting GLP-1, accelerated the hunt for a synthetic activator with a long half-life (requiring less than daily injections). Once we had such a long-acting peptide in hand, we needed to figure out how to use it. Ten years later, this remarkable class of peptides won the 2023 Breakthrough of the Year Award from the journal *Science* for its anti-obesity impact.

## THE STORY OF A DRUG

It started with the problem we have with sugar. Drug companies hoped these drugs would enable people with diabetes to get their hemoglobin A1c test results, the accepted metric for glucose control, down. If that were possible, then the adverse end-organ sequelae from diabetes, such as heart disease, could perhaps be prevented or at least delayed. Beginning in 2016, more than nine thousand people with type 2 diabetes were given the GLP-1 molecule liraglutide or a placebo. This drug trial resulted in fewer cardiovascular deaths, heart attacks, and strokes, even though it was associated with only five pounds of weight loss. One unsolved puzzle is that weight loss resulting from the drug in people with diabetes is substantially less than in those who are simply obese.

It took until 2021 for another GLP-1 (specifically the semaglutide peptide) to be tested on people with obesity, but not diabetes. That trial found a dramatic effect—an average loss of 15 percent of body weight, or thirty-four pounds. What was different in that experiment was the dose escalation to 2.4 milligrams of semaglutide, more than double the dose used in previous Ozempic trials. Novo Nordisk changed the name of the drug from Ozempic to Wegovy even though the semaglutide molecule was the same, just delivered at a higher dose. Then the new synthetic molecule tirzepatide, tested in a similar clinical trial, exceeded the weight loss achieved by semaglutide, inducing more than 20 percent body weight loss at higher doses. The manufacturer of tirzepatide, Eli Lilly, followed suit and changed the name of its diabetes drug from Mounjaro to Zepbound for obesity, even though, again, the only difference was the dose.

The naturally occurring GLP-1s and related gut hormones have receptors in the body that are activated by these peptides. The semaglutide peptide activates only the GLP-1 receptor peptide already in the body, whereas tirzepatide is a dual-receptor peptide, activating both the GLP-1 receptor and another gut hormone receptor called gastric inhibitory polypeptide (GIP). Now there are even triple-receptor activators such as retatrutide, which adds glucagon. By forty-eight weeks in a pivotal clinical trial, this agent, at doses of 8 or 12 milligrams, reduced weight by 24 percent, and there was no sign of plateauing. That means the weight loss may continue over time without established limits known to date.

These levels of body weight loss equate to or exceed what is typically observed with gastric-bypass surgery.

Imagine if the researchers and companies had expedited a longer-lasting GLP-1 drug by experimenting with higher doses from the get-go. This glaring miss might have benefited so many—the millions of people suffering the consequences of obesity, those with increased risk of heart attacks, heart failure, strokes, diabetes, high blood pressure, fatty liver disease and cirrhosis, Alzheimer's disease, sleep apnea, kidney failure, depression, cancer, severe osteoarthritis requiring joint replacement, and infertility. There aren't many chronic diseases that are left to put on this list. This breakthrough family of drugs, and future derivatives such as the pills, like orforglipron, or even a vaccine-like version, have extraordinary potential to promote health span beyond what they have already accomplished.

There are about a billion obese people on the planet, comprised of more than 650 million adults and 340 million ages five to eighteen years. That number, as defined by a body mass index (BMI) of more than 30 $kg/m^2$, has been growing for decades. Unfortunately, BMI is a flawed metric that both over- and underestimates obesity. It doesn't distinguish between muscle and fat, yielding abnormal results in athletes, while one in three people in the normal range is obese when body fat is measured. It is used pervasively, even though the easy-to-measure waist circumference and waist-to-height ratio would be more accurate and are the preferred metrics of obesity experts. We might even go to the technology more precisely measuring body fat percentage from a dual X-ray absorptiometry scan.

Nevertheless, using BMI in the United States in 2023, according to the Centers for Disease Control and Prevention, the prevalence of obesity among adults was above 40 percent and 20 percent in children and adolescents. The increase has been relentless: in 1990, the prevalence was 15 percent in adults and 10 percent in kids. By 1998, when obesity prevalence doubled, deaths from severe obesity-related heart disease tripled. People of color, those with low income, and those who live in rural regions of the country have even higher rates of obesity. It had reached staggering proportions with no potential remedy in sight—until the GLP-1 drugs arrived. If we add that another 30 percent of Americans are merely overweight rather than obese (BMI more than or equal to 25 $kg/m^2$) and may

stand to benefit, the transformative implications of this new medical technology become obvious.

So, we lost twenty years in the advance of public health by not noticing that the same drugs that could help people with diabetes could help those with obesity. A couple of decades might not seem much, given that obesity has been a growing problem in human health since the late eighteenth century. Apart from all the advances in medical science and human biology over those centuries, a key enigma was the role of hormones in weight gain, particularly the incretins, a family of hormones sending signals from the gut. When I finished medical school around 1980, we weren't taught that the gut could make hormones.

Incretin hormones, which include GLP-1 made from the cells lining the gut, are distinct from insulin and glucagon manufactured and released by the pancreas. Some of the signals discovered among the incretins run in a short-term system between the gut and brain, going in both directions. This information network is always busy sensing and regulating the calories we ingest. Other incretin hormones make up a long-term system regulating energy expenditure and the mass of fat tissue. This system assures that our fat tissue mass, our principal site of energy storage, is maintained. As a result, overeating or caloric restriction for a period is unlikely to induce a durable change in fat tissue mass. That's why so many diets have yo-yo results, because it's hard to override this tight regulation of our physiology—it's like the sodium or potassium levels in our blood, or core body temperature. The two systems, long and short term, are highly integrated. Discovering and observing how the gut-related hormones—such as ghrelin produced in the stomach, leptin from adipose tissue, amylin made in the pancreas, cholecystokinin and peptide Y from the intestine—operate in our bodies has dramatically changed our perspective.

When we eat sugar, insulin levels go much higher than if sugar is injected into our bloodstream. This had to be explained by something in the gut, but it was elusive. The first incretin hormone discovered was GIP in 1970, but its role in stimulating insulin secretion was not immediately obvious. Svetlana Mojsov, a Yugoslavian chemist who ran the Massachusetts General Hospital in Boston core facility, made the shortened GLP-1 (7-37) peptide, and proved that it triggered insulin secretion, unlike the full GLP-1 peptide. Subsequently, in 1986, Mojsov

and Habener published a paper that showed this form of the peptide occurred naturally in gut tissue. Svetlana Mojsov's story is another example of a long history of female scientists getting sidelined, such as Rosalind Franklin's pivotal participation in the discovery of the DNA double helix in the 1950s. In the case of GLP-1, Mojsov, after waging a legal battle to be listed as a co-inventor on key patents, ultimately prevailed in 2006. But that was long after many international prizes were awarded to Habener and collaborators.

I was involved in another related GLP-1 recognition—the Mani Bhaumik Breakthrough of the Year Award. In 2024, I served on a committee of the American Association for Advancement of Science to determine who was responsible for pushing these drugs into obesity clinical trials, despite the skeptics. After many interviews, much digging, and reviewing many documents translated from Danish, our committee recognized Lotte Bjerre Knudsen, chief scientific adviser in Research and Early Development at Novo Nordisk, and Richard DiMarchi, distinguished professor of biochemistry at Indiana University, as the two key players. Had it not been for them, we might still not know about GLP-1's pronounced impact on obesity.

It's fair to say that even though the GLP-1 family of drugs is having such pronounced effect on weight loss now, we don't fully understand why. For semaglutide, the single GLP-1 receptor activator, the effect is less than tirzepatide, a dual-receptor—GLP-1 and GIP—agonist. It's unclear as to what role the GIP activation has in increasing the magnitude of weight loss. This becomes more complicated when you consider that a molecule being pursued by Amgen, known as cafraglutide (MariTide), is a monoclonal antibody (man-made antibody) designed to bind to two gut hormone peptides, acting as an activator of GLP-1 but a blocker of GIP. In a trial with the drug given monthly (as compared to weekly for semaglutide and tirzepatide), it achieved substantial body weight loss, about 15 percent of body weight, at the highest doses, akin to semaglutide. The most potent GLP-1 family drug to date, retatrutide, one of the triple-receptor activators being developed, revs up the third, added glucagon receptor, which may reduce energy uptake or increase its expenditure, or both. There is one thing surprising about the drug class that is rarely seen: a dissociation between side effects and efficacy. Usually, they track together. A high proportion of people

taking these drugs in early weeks experience gastrointestinal side effects that fade. But the weight loss and anti-inflammatory effects do not. The reason for this unusual dissociation may be tied to the discovery of a distinct brain neural circuit that mediates the gastrointestinal side effects of nausea and vomiting in response to GLP-1 drugs. That may lead to a way to prevent or reduce these side effects in the future.

Altogether, these results don't add up to a simple explanation of how these gut hormones work. This is not so unusual in medicine. Many drugs, such as anesthetics routinely used during surgery, antidepressants, metformin for diabetes, aspirin, and lithium for bipolar disorder, are mysteries when it comes to the precise mechanisms that make them work.

But there are parts of the mechanism we do now understand. Before taking a bite of food, these drugs induce a sense of fullness, termed *pre-ingestion satiation*, mediated by neurons in the hypothalamus. We're familiar with postmeal satiety, but the discovery of two neuronal "fullness" circuits in the same part of the brain, one for pre-ingestion and the other after eating, both activated by GLP-1 drugs, helps provide an explanation for how these drugs work. The GLP-1 drugs also lower body weight in part by slowing stomach emptying, providing a sense of fullness with stretching of the stomach, signaling vagus nerves on its surface to fire, and relaying that information to the brain. The drugs can induce nausea, sensed in the brain, which of course also reduces appetite. Although GLP-1 drugs can get across the blood-brain barrier to some extent, they also talk to the brainstem and hypothalamus via the gut-brain axis, activating neural circuits that regulate appetite and reward. This modulation of part of the brain's limbic system dulls cravings and suppresses addiction to many things besides food: alcohol, tobacco, and gambling. There's also a noted shift in the foods eaten by people who are taking GLP-1 drugs, from less candy and snack foods to more vegetables and fish. As the pancreas secretes more insulin, muscles, fat tissue, and the liver happily become less resistant to insulin. These effects improve glucose control, which helps to fend off the pernicious effects of metabolic syndrome.

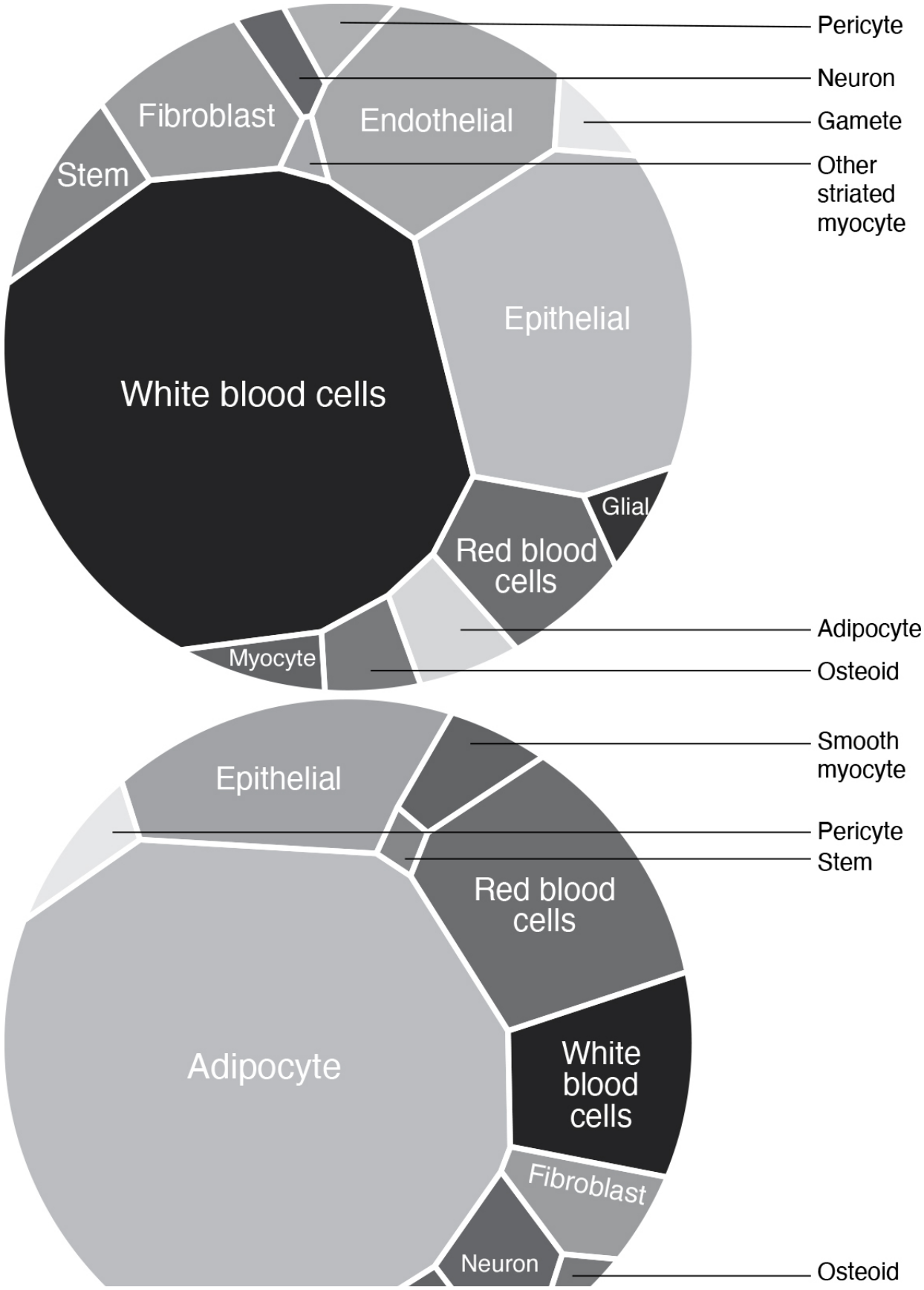
Metabolic syndrome is present in about two-thirds of people with obesity and is a marker of increased risk of heart disease, cancer, and neurodegenerative disease. It consists of five features (diagnosis of metabolic syndrome requires at

least three of these five features to be present): high fasting glucose (>110 mg/dl), high blood pressure (more than 130/85 mm Hg), high fasting triglycerides (more than 150 mg/dl), low high-density lipoprotein (HDL) cholesterol (below 40 mg/dl in men or <50 mg/dl in women), and central adiposity as defined by waist circumference more than forty inches in men or thirty-five plus inches in women. All are ameliorated in most people with obesity who take GLP-1 drugs. Because only three of the five features are needed to meet the diagnosis of metabolic syndrome, obesity is not a necessary component. We must flag that metabolic syndrome is prevalent in *one-third of people who are not obese*, thus further expanding the potential use of these drugs in the future.

GLP-1 drug reduces inflammation throughout the body and in the brain. Receptors to GLP-1 are plentiful in the brain, unlike other parts of the body. An elegant study that tested different GLP-1 drugs in mice whose GLP-1 receptors were erased from their brains showed that without these receptors, the anti-inflammatory effect was lost. Since there are no GLP-1 receptors in the cells of many organs, such as blood vessels and the liver, it questions how inflammation is suppressed by these drugs. Everything we know at this point implicates the GLP-1 impact on the brain as a key mediator for the body-wide reduction of inflammation. Recent work has shown that activation of GLP-1 receptors on T cells is another mechanism for the anti-inflammatory properties of these drugs.

## ADIPOSE CELLS AND TISSUE

Before we leave obesity and switch over to diabetes and metabolic health, it's worth looking closely at a few salient features of adipose tissue. It's composed of two main types of cells—white and brown. The white fat cells layer around our organs to provide protection, insulate our body heat, and store energy. In adults who are lean, white fat deposits account for 30 to 40 percent of total body mass in women, and 15 to 25 percent in men. Shown in figure 4.1 is the remarkable disparity in estimated cell count and mass in a lean male of about 150 pounds.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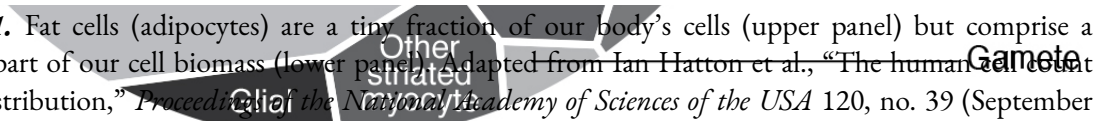



***Figure 4.1.*** Fat cells (adipocytes) are a tiny fraction of our body's cells (upper panel) but comprise a dominant part of our cell biomass (lower panel). Adapted from Ian Hatton et al., "The human cell count and size distribution," *Proceedings of the National Academy of Sciences of the USA* 120, no. 39 (September 2023): e2303077120, https://doi.org/10.1073/pnas.2303077120. Description 15

Adipocytes comprise a relatively tiny proportion of our thirty-seven trillion cells by count (upper panel above), but by mass they are overwhelmingly the dominant component, and almost all of that is white fat tissue.

In contrast, brown fat is a tiny fraction of our fat mass, only 0.2 to 3 percent; it consumes glucose and triglycerides to generate heat. Even that small amount declines with age and with increased ambient temperatures. White adipocytes secrete factors (adipokines) that communicate with organs including the brain. Some reduce sensitivity to insulin. Some activate the immune system. Most of these factors are pro-inflammatory, which is why I tell my patients that belly fat serves as the reservoir, the machinery, for promoting inflammation throughout the body. Indeed, decades of research on waist circumference has shown it is a highly informative metric and should be used as a vital sign in routine clinical practice, but sadly that has not ever caught on.

Obesity is simply way too much white adipose tissue. Unfortunately, unlike any other cell in our body, white fat cells can increase their volume by more than a factor of ten! And compared with all the other types of cells in the body, look how big (fig. 4.2) they are before that exceptional capability of an order of magnitude expansion.

In a study of people who were overweight or obese, the more brown fat tissue, the more they were metabolically healthy by a variety of metrics, including ability to generate heat after food intake. If only we could activate our relatively scant brown fat cells, as has been done in a small group of people with a β3-adrenergic receptor agonist (mirabegron), which led to less insulin resistance, more insulin secretion, and better blood lipid results such as higher HDL and ApoA1. Perhaps we'll see more work to take advantage of the switch from white to brown fat in the future. In the meantime, albeit in small quantities, there's a third cell type in fat tissue—beige cells—that are scattered within white fat tissue and can be switched into brown with exercise or need for heat production. Exciting recent discoveries of a molecule that controls the size of brown and beige adipose tissue,

and conversion of white to beige fat, paves the way for a drug that will prevent age-related decline.

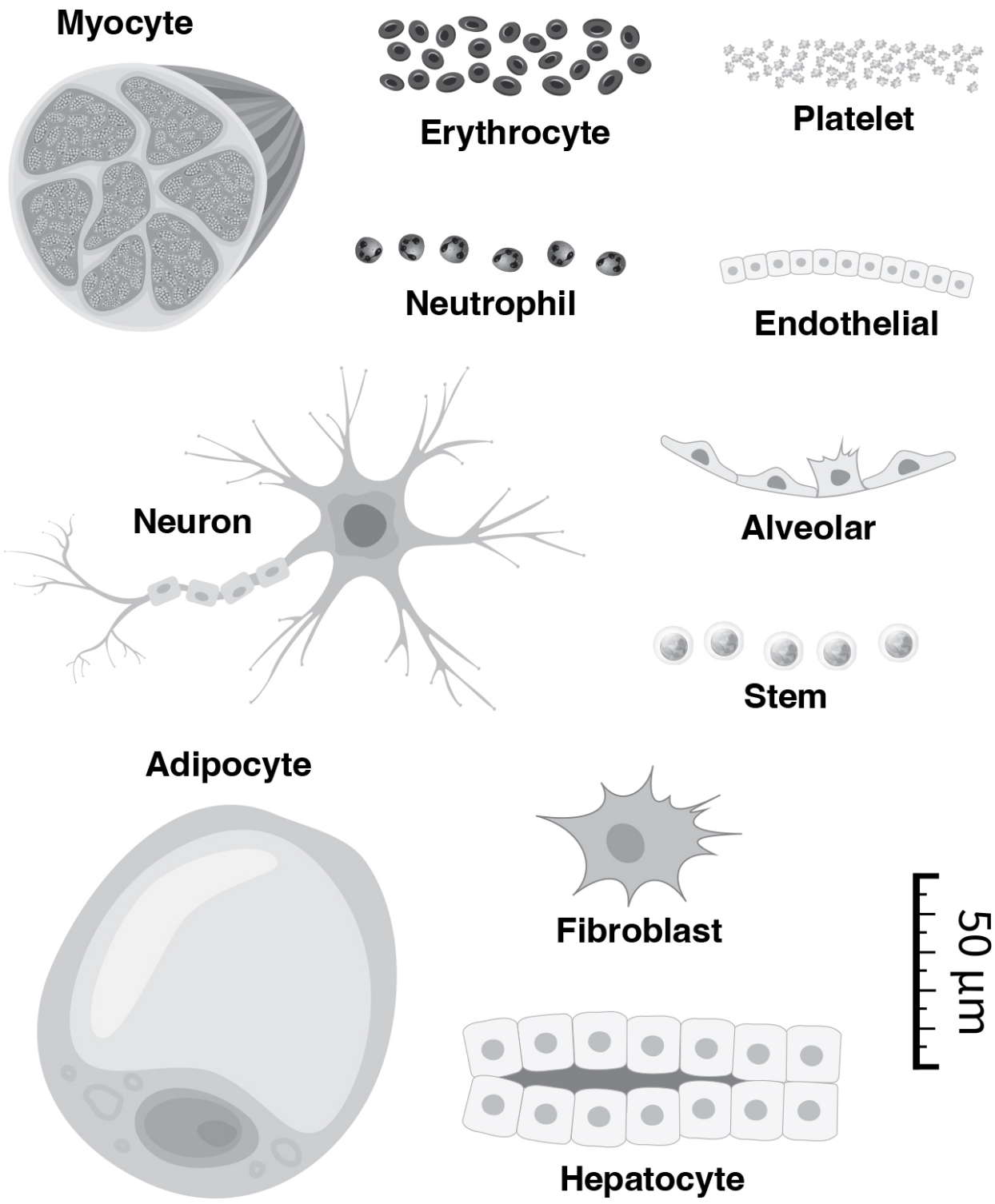



***Figure 4.2.*** Schematic of structures of major cell types in the body at scale. Adapted from Ian Hatton et al., "The human cell count and size distribution," *Proceedings of the National Academy of Sciences of the USA* 120, no. 39 (September 2023): e2303077120, https://doi.org/10.1073/pnas.2303077120. Description 16

The inflammation that is associated with white adipose tissue occurs throughout the body. In the brain, it's characterized by activation and recruitment of microglia, the resident immune cells. In the liver, fat tissue, and skeletal muscle, there's recruitment of macrophages, the innate immune cells, and from the bone marrow release of monocytes into the blood. This coordinated meta-inflammation is the essential biology underpinning of obesity and why it was reclassified as a complex, chronic disease.

Our diet is a key factor for promoting or reducing inflammation. For type 2 diabetes, the benefit of low-carb diets, with 10 to 25 percent of calories from carbs, or even lower, has been amply validated. A systematic review of twenty-three trials indicated a 32 percent increase in remission of diabetes. The intake of milk in people with lactose intolerance (as determined by a lactase LCT gene variant) has been linked to a favorable modulation of the gut microbiome and lower risk of type 2 diabetes. A relationship between the polygenic risk score for type 2 diabetes and titration of risk with a healthy diet has been established in multiple cohort studies.

## THE GOOD AND BAD NEWS FOR PUBLIC HEALTH

Historically, obesity was stigmatized. We fat-shamed people because they supposedly didn't have enough willpower. In 2020, a joint international consensus called for ending the stigma of obesity, and we have since seen obesity increasingly recognized as a chronic disease. It transcends lifestyle factors like nutrition and exercise, and is influenced by genetics, the gut microbiome, the person's environment, and physiological and socioeconomic factors. We've recently learned that the response to GLP-1 drugs can be variable, and there are at least some genetic variations in the receptor that have been discovered that help explain why that's the case. The researchers who made this discovery suggest the GLP-1 receptor variants should be assessed before using these drugs in people with diabetes. Over 10 percent of people who take GLP-1 drugs for more than a year lose less than 5 percent body weight. A genetic test predicting how well—or not—these drugs will work for someone with obesity is now available.

The significance of the GLP-1 science breakthrough can be appreciated by the long history of failures that preceded it. In the 1990s, a combination drug of two neurotransmitter releasing agents—fenfluramine and phentermine—known as fen-phen, was widely prescribed, even without FDA approval, after a randomized trial showed it achieved an average weight loss of 18.5 pounds compared with 10 pounds in the placebo group. There were over eighteen million prescriptions of the combo in 1996. Soon case reports appeared of people who took fen-phen and then developed valvular heart disease and hypertension of the lung blood vessels (known as pulmonary hypertension). By 1997, the Mayo Clinic documented this occurrence in twenty-four young women, average age of forty-four years, alongside many other reports to the FDA, which led to its withdrawal later that year. Later, a systematic assessment of the drug pointed to about three people in ten developing fen-phen-induced valvular heart disease. This wasn't the only new drug strategy that backfired with serious safety concerns.

Soon after, there was excitement about the anti-obesity drug rimonabant. It was directed against the cannabinoid receptor in the brain—the same receptor that induces the munchies after smoking or ingesting pot. Multiple reports showed that high-dose rimonabant helped people lose fifteen pounds of body weight, and they had better lipid panel results, lower fasting glucose, and less resistance to their insulin. Keen interest led to a major international trial (which I ran) to see if the drug could reduce heart attacks and strokes. Close to nineteen thousand participants from forty-two countries joined to take the active pill or placebo for three years. At the same time the clinical trial was ongoing, rimonabant had already been approved by regulators in some countries to achieve weight loss. Then came the bad news: reports of suicides or suicide attempts were clustering, both in the trial and in several European countries. Rimonabant was withdrawn from the market in 2008, and our trial had to be discontinued after one year of follow-up, with four suicides in the treatment group and one in the placebo group. Sanofi, the company that developed the drug, stopped its production and commercial pursuit. Meridia, an appetite suppressant (sibutramine) anti-obesity drug marketed by Abbott Laboratories, was withdrawn in 2010 because it significantly increased the risk of heart attacks and strokes. Other nondrug strategies in recent decades to reduce obesity, such as the

Atkins high-fat diet craze or imposing soda beverage taxes, both failed. At least they didn't result in heart disease or suicide.

Given that background, the success here is all the more impressive. We have never seen a class of drugs capable of this magnitude of weight loss. That alone would be considered a monumental achievement, but the impact these drugs are having on heart, kidney, and liver disease goes beyond that achievement.

The cardiovascular benefits of GLP-1 drugs in people with obesity have been measured in two ways. In a large randomized trial of 17,600 people with a high BMI (average of 33 $kg/m^2$) and history of heart disease, treated with semaglutide or placebo for three years of treatment, there was a 20 percent relative reduction, or 1.5 percent absolute reduction, of heart attacks, strokes, and cardiovascular deaths. Of note, there was a substantial reduction of inflammation, as reflected by the biomarker high-sensitivity C-reactive protein (hs-CRP), and the adverse cardiovascular events, before there was substantial weight loss. These results emphasize the GLP-1 effect on reducing inflammation that *precedes and is independent of* weight loss.

In two randomized trials of a certain type of heart failure (preserved ejection fraction) that accounts for approximately half of all cases of heart failure, there was a marked reduction of symptoms and improvement in how much exercise people were able to do. One of the trials was in patients with diabetes and obesity; the other was obesity alone. Once again, the weight loss was substantially less—nearly half as much—in people with diabetes. Notably, GLP-1 led to a substantial decrease of hs-CRP that emerged *before* the effect on weight loss. That, along with weight loss, may account for the marked reduction of atrial fibrillation in multiple randomized trials of GLP-1 drugs.

The benefit of GLP-1 for kidney function was assessed in over thirty-five hundred people with diabetes and obesity with impaired renal function. The effect of the drug was so beneficial that the trial was discontinued early. A dose of 1 milligram of semaglutide dramatically reduced kidney failure, the need for dialysis or kidney transplant, and even death from cardiovascular causes.

While not as common as obesity, nonalcoholic steatohepatitis causes one in four people worldwide to develop fatty liver disease (NAFLD) and 10 percent of these progressing to the severe form (NASH), with marked scarring (fibrosis),

cirrhosis, and liver cancer. Ten to 20 percent of cases are in lean, nonobese individuals. These conditions have been renamed to MASH and MALFD, with the *M* standing for metabolic dysfunction to separate them from obesity and alcohol-related ailments. The first approved drug for MASH is resmetirom (Rezdiffra), approved in 2024, which improves mitochondrial function in liver cells. Multiple GLP-1 drugs are showing benefit, including semaglutide in patients with human immunodeficiency virus (HIV) and MALFD, as well as randomized trials of tirzepatide and survodutide for patients with MASH. Prevention of the fibrosis and its progression is the key determinant of outcomes. By achieving improved glucose control and some weight loss in people with type 2 diabetes, there are studies in people that suggest GLP-1 drugs may help fix this specific metabolic dysfunction.

The GLP-1 anti-inflammatory effect in the brain has led to ongoing clinical trials for prevention of both Parkinson's disease and Alzheimer's disease. For Parkinson's, there have been three small randomized trials of GLP-1 drugs versus placebo, with a consistent benefit of stopping or reducing progression of motor symptoms. A real-world study of nearly ninety thousand Medicare beneficiaries over four years who were new users of a GLP-1 drug compared with dipeptidyl peptidase 4 (DPP-4) inhibitors drugs found a 23 percent lower risk of developing Parkinson's disease. To put this in context, we have never had a drug that is disease modifying for Parkinson's disease; the only medications available treat symptoms such as tremor and rigidity.

A study in Sweden of more than eighty-eight thousand participants aged sixty-five years and older with type 2 diabetes compared the rate of developing dementia for treatment with either GLP-1 drugs, DPP-4 inhibitors, or sulfonylureas during four-year follow-up. Like the effect seen with Parkinson's in a similar study, the GLP-1 drugs were linked to a 30 percent less risk of dementia. A twelve-month randomized trial of liraglutide versus placebo for participants with mild to moderate Alzheimer's showed benefit for both cognitive function and brain imaging. A small randomized trial of a GLP-1 drug in early Alzheimer's disease slowed cognitive decline and reduced brain shrinkage of key regions compared with placebo. There is work in progress on an intranasal GLP-1

delivery to improve distribution to the brain. Two large randomized trials of semaglutide in patients with early Alzheimer's are expected to report out in 2026.

Almost forty million Americans have sleep apnea. The favorable impact of GLP-1 drugs for treating sleep apnea in people with obesity was documented in two randomized trials of tirzepatide. Along with the marked weight loss, there was substantial reduction of apnea (nearly 60%), oxygen desaturation in the blood, patient-reported sleep impairment and disturbance, systolic blood pressure, and the inflammation marker C-reactive protein. Notably, all these improvements occurred in patients using or not using continuous positive airway pressure (CPAP) machines. The results have put in question the relative need for CPAP in the future, a device that many patients love to hate, or refuse to use.

What about GLP-1 drugs affecting cancer? While there are no randomized trials to draw from, in a study of 1.6 million people with type 2 diabetes, among those treated with GLP-1 drugs compared with insulin, there was reduction in eleven of thirteen cancers that are associated with obesity. Separately, from a study of 1.2 million people with type 2 diabetes, there was a marked reduction of colorectal cancer in those taking GLP-1 drugs compared with either insulin or metformin, even in those who were not overweight or obese. It's harder to attribute these findings to weight loss, which is much less pronounced among diabetics. Perhaps it is a result of the direct anti-inflammatory effect or the known activation of T cells with antitumor activity in preclinical models.

Several reports suggest that GLP-1 drugs have other diverse benefits, such as a 50 percent reduction in alcohol intake; enhancement of fertility in women with polycystic ovary syndrome ("Ozempic babies"); reduction or cessation of tobacco abuse, gambling, and nail-biting; and treatment of autoimmune disorders such as psoriasis, arthritis, and asthma as well as depression. You'll note that many of these conditions have little to do with obesity or weight loss. And yes, antiaging properties have also been identified. Simply put, it's hard to find a possible indication that hasn't been raised for GLP-1 drugs.

The news about GLP-1 drugs is not all good. In the trial with the longest follow-up at high doses (to almost forty months), twice as many people permanently discontinued the drug (16.6%) compared with placebo (8.2%). The main reason was the fivefold increase in gastrointestinal disorders that include

nausea, diarrhea, vomiting, constipation, and abdominal distension, which typically occur early after treatment initiation, and rare cases of acute pancreatitis, acute gall bladder disease, and gastroparesis (slowing or cessation of food moving from stomach to intestine). Resting heart rates increase on the drugs; though usually only by a few beats per minute, in some people this can be substantial, up to twenty beats per minute. Blurred vision and exacerbation of diabetic retinopathy are known risks, even though the latter would be expected to be reduced over the longer term with better glucose control. More serious is one report of a higher incidence of a type of optic neuropathy (nonarteritic anterior ischemic optic neuropathy, which can result in sudden vison loss, frequently called an "eye stroke") in an observational study of people taking semaglutide compared with matched controls. But other studies have found just the opposite, so this risk remains uncertain. It also goes against the anti-inflammatory properties of GLP-1 drugs, but time will tell if it is a risk rather than a spurious association. Anesthesiologists have raised the concern that patients undergoing surgery should not take their GLP-1 medication for seven days prior to reduce the problem with vomiting and aspiration pneumonia, but this is not resolved. The drugs are contraindicated in people with rare forms of cancer: medullary thyroid carcinoma or multiple endocrine neoplasia syndrome type 2.

And that's not all. There is consternation about GLP-1-induced loss of muscle mass and the potential development of "sarcopenic obesity" (meaning general loss of muscle mass and strength). Reports are mixed, with some suggesting the incidence is as high as 40 percent and others using magnetic resonance imaging claiming it's a nonissue. It is confounded by the fact that significant weight loss without medication also leads to reduction in skeletal muscle mass. No matter, loss of muscle mass does occur in many people, and some have advocated an increased dietary ingestion of protein and strength training to counter these possible side effects. Nevertheless, this remains an important issue. The companies that make these drugs are acquiring or partnering with companies that have drugs in clinical trials with GLP-1 drugs to preserve or increase muscle mass, such as bimagrumab, apitegromab, or azelaprag, and many others are in development. In the meantime, those considering starting

a GLP-1 drug may want to get a baseline DEXA scan in order to track their muscle mass.

While there have been concerns about loss of bone density with GLP-1 drugs, a randomized trial in people with increased risk of fracture reported the changes in bone turnover and mass was due to the loss of weight and mechanical loading, not the drug. Results from another randomized trial reinforced the benefit of exercise while taking GLP-1 drugs for achieving optimal bone health metrics.

In my mind, the biggest drawback of GLP-1 drugs—"forever drugs"—is the potential need for a lifetime commitment. For whatever reason, it is difficult to follow through on that commitment. Most people taking GLP-1 drugs stop them in the first year. Randomized trials have shown that stopping the drugs leads to regaining the weight, and we have not seen any commitment from the manufacturers to identify or test an exit strategy. While the current dependence on once-a-week injections will be replaced by pills that simulate the GLP-1 effect in the near term, that still means lifelong therapy. Colleagues of mine who specialize in bariatric medicine say that's fine—it's just like giving insulin to a diabetic or blood pressure medicine to a person with high blood pressure. While the analogy is easy to understand, there are still unknowns about GLP-1 drug side effects at high doses in the long term—the longest follow-up we have for obesity is four years. If the risk of sarcopenic obesity is real, then the number of frail people with increased risk of falling would substantially increase. What's even more disconcerting is that much of the obesity pandemic is in children: an indeterminate, and perhaps lifelong, commitment to taking a drug from a young age is troubling, to say the least.

Beyond the issue of side effects, there is cost, which is high. In the United States, thirty days of Wegovy costs $1,350, although the company claims the copay for those with insurance only amounts to $25 per month out of pocket. The monthly tab is $147 in Canada and $93 in the United Kingdom. It has been pointed out the cost of making a month's worth of these drugs is about $5.

The problem with the cost of these drugs, coupled with the potential need for lifelong duration, is that the people with the most to gain will likely be the least to derive the benefit—again, obesity is most common among people of color and low socioeconomic status. The likelihood of them having insurance that covers

these drugs is quite low, no less access to physicians to get prescriptions. While the GLP-1 drugs have such formidable impact to promote health span, they may ultimately be viewed as the worst offender for taking health inequities in the United States to an unprecedented high.

## EVEN WORSE THAN OBESITY

Diabesity is a portmanteau term coined in 1973 by Ethan Sims following experiments in overfeeding twenty-four prisoners, increasing their body weight by more than 20 percent, and noting their development of glucose intolerance and insulin resistance. Little did he know that term, over the subsequent half century, would come to refer to the twin pandemics of diabetes and obesity. While most people who have type 2 diabetes are obese, there are many holes in that relationship. Many thin people can develop this type of diabetes; most overweight or obese people do not. Why?

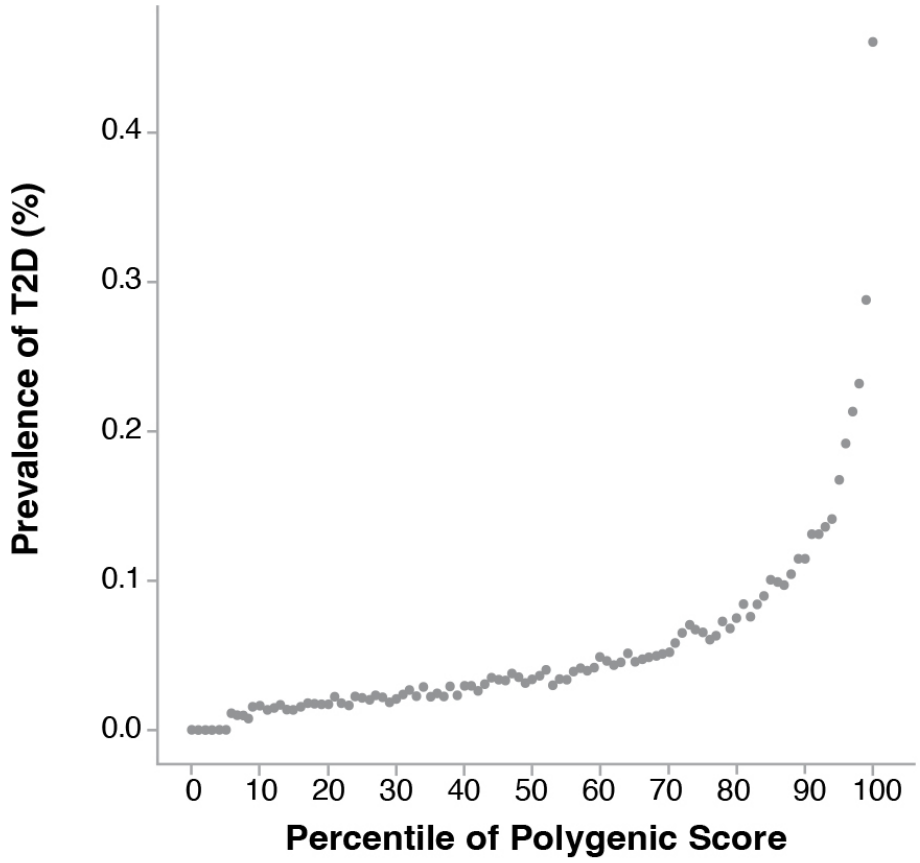
In the past two decades, the genetic underpinnings of type 2 diabetes have become clearer. "Type 2" is a mosaic of genes and pathways that include impaired insulin secretion, insulin resistance, abnormalities of insulin signaling or mutations in insulin receptors, pancreatic beta cell dysfunction, glucose transporter genes, and many more. It's not simple. There's considerable overlap from one person to the next, so we can't break it down easily to type 3, 7, 10, and so on. So we're stuck with this catchall of type 2. There is considerable heritability of type 2 diabetes that we can determine from a sample of a person's saliva or blood, using a gene chip (array) or a genome sequence. Hundreds of common genome variants are collectively linked to the risk of people developing type 2 diabetes. One's tally of these variants is known as a polygenic risk score, now validated across the main ancestries of European, African, Hispanic, and Asian.

The graphs in figure 4.3 show the relationship of the polygenic risk score, by percentile, from zero to one hundred, for prevalence of type 2 diabetes in over three hundred thousand people in the UK Biobank (upper panel) and the odds ratio for developing type 2 diabetes among over two hundred thousand people in the Million Veterans Program (lower panel). Remember that type 2 diabetes is

fundamentally different from its type 1 autoimmune diabetes counterpart, which is discussed in the chapter on autoimmune diseases.

The graphs in figure 4.3 are consistent and tell us that if one has a high score—90 or above—for the hundreds of genomic variants linked to type 2 diabetes, the risk of developing it is considerably increased. I've put both graphs in sequence to illustrate the difference between absolute risk (upper) and relative risk (lower). People with risk scores below 90 have only a small absolute risk, even though the odds ratio looks high. You can see how this absolute prevalence drops substantially below the 90 and 80 percent risk score (upper panel), whereas the big jump in relative risk is seen in the top 10 percent of polygenic risk score (lower panel).

Why is this important? For one, it helps explain why people who are not overweight or obese may still be at substantial increased risk for developing type 2 diabetes. For example, many thin people of Asian ancestry are prone due to their genetic makeup. About one of three people in the United States who are considered "pre-diabetic" and exhibit metabolic syndrome (vide infra), with abnormal fasting blood sugar (>110 mg/dl), are not obese. Whatever your body weight status, knowing your risk of diabetes can help promote its prevention, be it by changes in nutrition, exercise, and lifestyle, or with medications. Getting your polygenic risk score should not be expensive or difficult. The data can be derived from a gene chip (array) used by companies like 23andMe or AncestryDNA or low-pass genome sequencing. Either method detects the several hundred letter variants among the three billion letters of the human genome, and a formula is used to calculate the score, with weighting of specific variants by their importance, and critical attention to the person's ancestry.


Prevalence of T2D (%)
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
100
Percentile of Polygenic Score

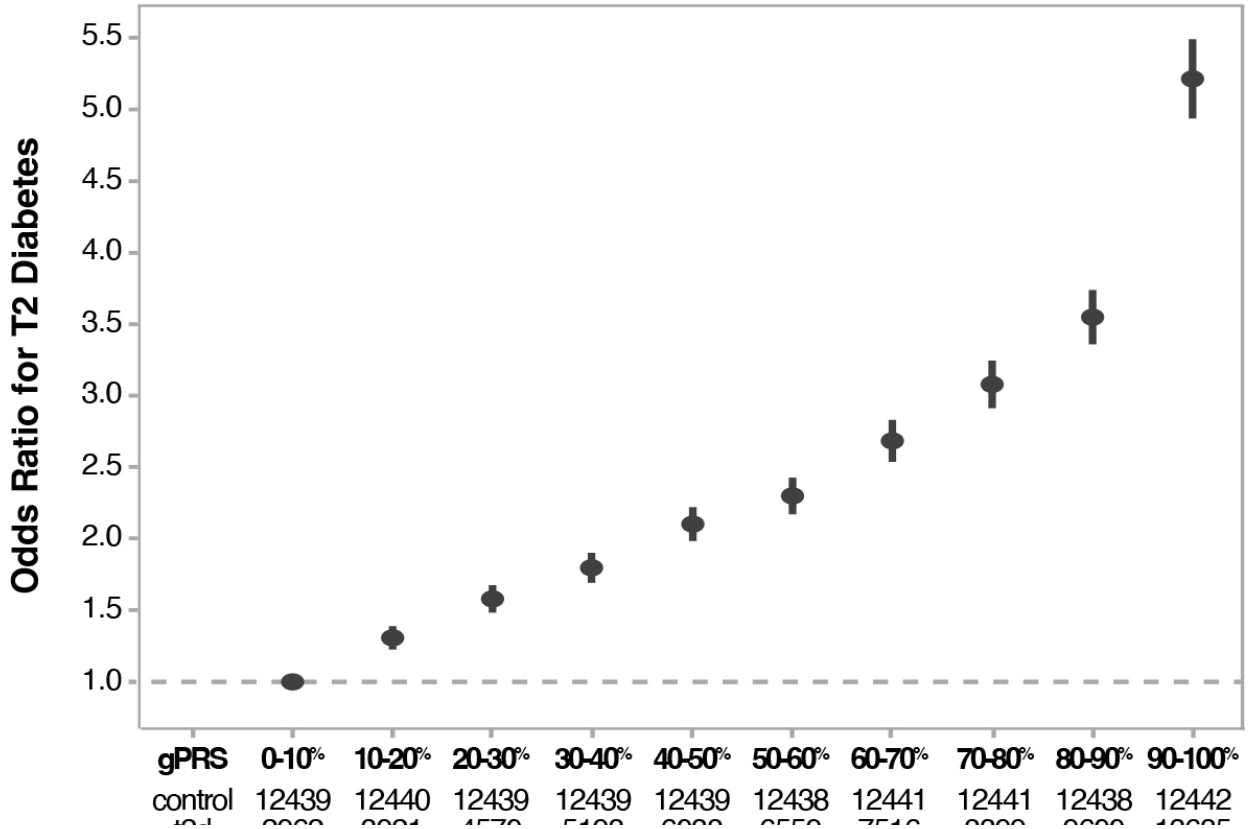

Odds Ratio for T2 Diabetes
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
gPRS
0-10%
10-20%
20-30%
30-40%
40-50%
50-60%
60-70%
70-80%
80-90%
90-100%
control
12439
12440
12439
12439
12439
12438
12441
12441
12438
12442

***Figure 4.3.*** Polygenic risk score and type 2 diabetes. Absolute increase with higher score (upper panel) and relative increase (odds ratio) of risk with increasing score (lower panel). Adapted from Wei Liu et al., "An improved genome-wide polygenic score model for predicting the risk of type 2 diabetes," *Frontiers in Genetics* 12 (February 2021): 632385, https://doi.org/10.3389/fgene.2021.632385 (upper panel); and Marijana Vujkovic et al., "Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ancestry meta-analysis," *Nature Genetics* 52, no. 7 (July 2020): 680–91, https://doi.org/10.1038/s41588-020-0637-y (lower panel). Description 17

Of note, the Mass General Brigham health system in Boston has started implementing array-based polygenic risk scores in their patient population for more than thirty-six thousand multiancestry participants. They found 8.4 percent fit into a high-risk category for type 2 diabetes, with an overall odds ratio of 1.75, and 1.93 for those of European ancestry. Nearly one in ten with nearly double the risk! Other studies have shown how blood proteins predict risk of type 2 diabetes better than a polygenic risk score of Hemoglobin A1c. Such informative data about high risk should be made available to everyone since there are so many ways to prevent this condition. Once type 2 diabetes takes hold, it is difficult, but not impossible, to turn the table back and achieve durable normal glucose control. The severity and chronicity of the disease is linked to many end-organ complications that include retinopathy, heart and vascular disease, kidney disease, strokes, neuropathy, increased risk of cancers, and Alzheimer's disease. The burden of diabetes was quantified by a population study of forty-six million people in England: "By the age of 50 years, one-third of those with diabetes have at least three conditions, spend more than 20 years with them, and die 11 years earlier than the general population." Simply put—nothing good. Even worse than obesity.

Your family history of type 2 diabetes is a factor, but your polygenic risk score provides valuable information because it is possible that the combination of variants you derived from your mother and your father is quite different from the set of variants either parent had alone, or their lineage. The hundreds of gene variants associated with type 2 diabetes are quite different from those linked to obesity, which adds to the notion that the biologic underpinnings for these two chronic conditions are distinct, and that type 2 diabetes can occur as a consequence of obesity, or on its own.

So much for the genomics of type 2 diabetes, let's move on to its treatment. In all of medicine there is no malady with a longer list of medications. Last count it had fifteen classes of them, including metformin, sulfonylureas, meglitinides, amylin analogs, alpha-glucosidase inhibitors, thiazolidinediones (TZDs), dipeptidyl peptidase (DPP-4) inhibitors, dopamine-2 agonists, bile acid sequestrants, insulin, symlin, pramlintide, sodium-glucose cotransporter-2 (SGLT-2) inhibitors, and GLP-1 receptor (e.g., semaglutide) or dual GLP-1/GIP agonists (e.g., tirzepatide).

Many of these are old, out of favor, or are not especially useful, but it gives a sense of how much attention has been paid to this condition. Only in recent times have we learned that one in four people with difficult-to-control type 2 diabetes has high levels of cortisol and may benefit from treatments such as mifepristone, the same medication used for emergency contraception. Note also that some diabetologists and endocrinologists devote their careers to it, alongside the primary care physicians who care for most with the disease.

Attempts to lose weight and improve nutrition and exercise, while important, will not typically achieve long-term normalization of glucose regulation (hemoglobin A1c). The classic first line of treatment, which also works to help prevent type 2 diabetes, is metformin, a well-tolerated, inexpensive, widely available drug. The mechanism for its benefit, as pointed out earlier, is elusive, but it has been shown to decrease liver production of glucose and improve muscle sensitivity to insulin. Unlike many of the drug classes on the list, metformin is not linked to risk of hypoglycemia.

Thinking as a cardiologist, there are only two classes of T2D drugs that should be given high priority because they not only markedly improve glucose control but also have been proven through large randomized trials to protect against heart attacks, strokes, and death. Those are the SGLT-2 inhibitors that block reabsorption of glucose in the kidney, facilitating its excretion in the urine, and the GLP-1 family of drugs. In fact, if it weren't for the cost, a recent analysis suggested they should be first-line treatment, not metformin or lifestyle intervention. These two classes of drugs, as a consequence of reducing end-organ damage, increase life expectancy. The SGLT-2 inhibitors, offering about a 15 percent reduction of death in people with type 2 diabetes or heart failure, are also

linked to fewer kidney stones, an age-related condition, and in the mouse model to enhance the elimination of senescent cells, extending lifespan.

It is another example of buying health span: expensive drugs with superior outcomes that outclass many low-cost, generic, "old" drugs. However, at the individual level, there is the potential for the patient to help drive the physician's decision for treatment of choice, at least in individuals with health insurance, which generally covers these preferred agents. Since their mechanisms for achieving better glucose control are different and don't overlap, it is not surprising that the combination of GLP-1 drugs and SGLT-2 inhibitors add to each other's effects. They may even be synergistic—adding up to more than their sum for reducing heart disease. A meta-analysis of twelve randomized trials involving more than seventy-three thousand participants with type 2 diabetes showed additive benefit for the combination of these two drug classes to reduce heart and kidney disease. The economics cry out for attention. We have more than thirty million Americans diagnosed with type 2 diabetes, and the cost of their care annually exceeds $413 billion. That figure doesn't include the approximately nine million people who have type 2 diabetes but remain undiagnosed.

## METABOLIC SYNDROME MESSAGING

Metabolic syndrome and prediabetes are two overlapping but different conditions, and both predict increased risk of type 2 diabetes. Even if diabetes doesn't manifest, they both can lead to cardiovascular disease and premature death. As seen in the table below for metabolic syndrome, and similar to the profile of prediabetes, neither is simply about how much one weighs; a substantial proportion of the people at risk are not obese.

The diagnosis of prediabetes is purely based on glucose metrics: either a fasting blood glucose of 100 to 125 mg/dL or Hemoglobin A1c between 5.7 and 6.4 percent. Diabetes is diagnosed by a fasting glucose of 126 mg/dL or higher, a random blood glucose greater than 200 mg/dL, or Hemoglobin A1c more than 6.4 percent. Normal is below 100 mg/dL fasting glucose and less than 5.7 percent Hemoglobin A1c. According to the Centers for Disease Control and Prevention,

about one hundred million Americans have prediabetes. The higher the Hemoglobin A1c, the more risk of heart disease.

On the other hand, the diagnosis of metabolic syndrome relies on at least three of the five conditions of the cluster to be present, as reviewed earlier (high blood pressure, high fasting blood glucose of 100 mg/dL, abnormal waist circumference, low HDL, and high triglycerides), only one criterion overlaps with prediabetes. Accordingly, a significant proportion of people diagnosed with metabolic syndrome may not have the abnormal fasting blood glucose. Ironically, however, the number of Americans who have metabolic syndrome is also about 100 million, as calculated from the 2020 National Health and Nutrition Examination Survey data, with an adult population of 255 million people. Note in the table below that the 100 million adults with metabolic syndrome are almost equally divided between obese and nonobese.

| Prevalence of Obesity and/or Metabolic Syndrome in American Adults | |
|---|---|
| **Category** | **Number of Americans (in millions)** |
| Obese with metabolic syndrome | 50.9 |
| Obese without metabolic syndrome | 31.8 |
| Nonobese with metabolic syndrome | 49.1 |
| Nonobese without metabolic syndrome | 123.4 |

(Adapted from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6484904/ https://www.cdc.gov/nchs/about/factsheets/factsheet_nhanes.htm https://academic.oup.com/pmj/article/99/1175/985/7076129)

Counter to what many would anticipate, the highest risk of death among people with metabolic syndrome is not in the people who are obese; it's those with normal weight and overweight, as seen in a Centers for Disease Control and Prevention three-year follow-up study. While those with normal weight had the highest mortality, people who were obese carried substantially more risk than those who didn't have metabolic syndrome at all (fig. 4.4). Not all studies agree with this observation of highest risk in those with normal weight, but it has raised awareness for the importance of diagnosing metabolic syndrome in people

irrespective of their body weight, highlighting that factors not related to body weight (such as waist circumference) are critical components of the diagnosis.

There has also been the question raised about the category of "metabolically healthy, obese." That is quite unfortunate, since there is no evidence that the condition of obesity can be viewed as being healthy, even without metabolic syndrome. Debunking that notion, many studies have demonstrated that people in this category should not be considered safe.

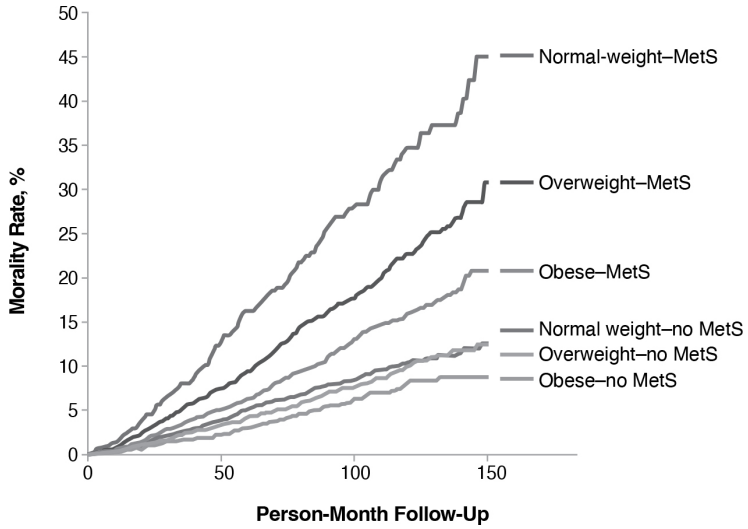



***Figure 4.4.*** Mortality risk associated with Metabolic Syndrome (MetS) and weight status. Adapted from Ting Huai Shi et al., "The influence of metabolic syndrome in predicting mortality risk among US adults: Importance of metabolic syndrome even in adults with normal weight," *Preventing Chronic Disease* 17 (May 2020): E36, https://doi.org/10.5888/pcd17.200020. Description 18

Similar confusion surrounds prediabetes. In the journal *Science*, the investigative reporter Charles Piller wrote about a frontal assault on the classification of prediabetes, claiming it was scaremongering, invented by a public relations officer for the American Diabetes Association. Piller went on to highlight the financial conflicts of this organization and the outspoken researchers who have advocated diagnosing prediabetes as well as the potential gain of pharmaceutical companies to offer drugs for this diagnosis. While many of these points were valid, prediabetes is real and has since been shown to be linked to heightened risk of heart attacks, strokes, and death.

What's more, improvements in lifestyle, and to a lesser extent taking metformin, have been shown to mitigate this risk. Like suggesting that obesity

can be healthy if in some narrow sense your metabolism is, questioning whether prediabetes is worthy of diagnosing was a bad public health message.

Instead of trying to assuage concern for people with metabolic syndrome or prediabetes, we should be sounding alarms. These are red-flag warnings that a person carries a high risk for progressing to chronic disease that has serious secondary consequences. And we're better positioned now to intervene without relying solely on hard-to-adopt lifestyle changes. A big step in achieving maximal health span is maintaining metabolic health. Keep in mind that only 7 percent of Americans fulfill the definition of being metabolically healthy.

Let's circle back to the GLP-1 drug breakthrough. Since these drugs have anti-inflammatory effects and improve metabolic biomarkers, including better glucose control, promoting sensitivity to insulin, achieving a more favorable lipid profile, and modifying behavior, their potential applicability for metabolic syndrome and prediabetes is clear, yet not adequately tested.

## THE WAY OF MEDICAL TECHNOLOGY

Just being highly effective drugs for obesity and diabetes, and preventing their complications, GLP-1s would have been considered a major medical triumph. But now it seems possible, especially with increasingly potent triple-receptor agonists, available in pill form, that the GLP-1 family of drugs will offer far more. It is conceivable that most people will be taking one of the GLP-1 drugs in the future.

Only one comparable breakthrough class of medications seems obvious to me, cardiologist that I am—statins. They are taken daily by an estimated two hundred million people around the world, with considerable regional variability by income status of countries. About fifty million people in the United States take a statin, even though its population only accounts for 4.2 percent of the world. That huge number of statin takers is in the same ballpark as the number of Americans who are currently eligible for GLP-1 drugs. There are many parallels between GLP-1s and statins from which we can learn.

I love the term *pleiotropic*, which is frequently used in medicine to explain things we don't understand. Instead of just fessing up that we don't know, this

fancy word makes it seem more scientific—that the medication has multiple effects, and we don't know which one, or some combination of them, that accounts for the benefit. This is quite true for both statins and GLP-1s. Nevertheless, they both feature a reduction of inflammation, as seen by biomarkers like hs-CRP. And inflammation is the final common pathway for most chronic diseases, spanning from cardiometabolic to neurodegenerative.

It is hard to parse out that impact from other effects: such as lowering LDL cholesterol by statins or improving glucose control by GLP-1s. What's surprising is that both classes of drugs have substantially unknown mechanisms for most of the good they do. (Meanwhile, we tend to insist on explainability of artificial intelligence before it can be used in health care, holding it to higher account than we do everyday treatments.)

The expectations for GLP-1s in 2023, after showing reduction of heart and kidney disease, became outlandish. Soon after positive results from these clinical trials, financial analysts projected that we are at the beginning of *the end* of obesity, heart disease, type 2 diabetes, sleep apnea, and kidney disease. This exuberance is understandable but irrational. Sure, there might be billions of people who could benefit from GLP-1 drugs, but the number of people who will take them is only a tiny fraction. That's not just because of cost, access to prescription, and limited supply but also because many people can't tolerate the drugs due to their gastrointestinal side effects. Likewise for statins, there are a substantial proportion who get muscle aches and cramps, intolerance that leads to discontinuation.

The first statins—lovastatin (Mevacor) and simvastatin (Zocor)—were commercially released soon after I started practicing cardiology in the mid to late 1980s. I've had the chance to watch their transformative but incomplete impact over the next four decades. A study reported in late 2023 demonstrated that the proportion of guideline-eligible adults taking statins plateaued at only 35 percent over two decades. These were people who should be taking a statin for primary prevention, meaning they had a high LDL cholesterol but no cardiovascular disease, type 2 diabetes, or a high-risk score for heart disease. Even among people with an LDL greater than 190 mg/dL, which is exceptionally high, less than half were taking a statin.

But that didn't stop statin exuberance. Michael Brown and Joseph Goldstein, who were Nobel laureates for their work on the LDL receptor that led to statins, wrote "Heart Attacks: Gone with the Century?" forecasting that statins "may well end coronary disease as a major public health problem early in the next century." Nearly three decades later, while there has certainly been progress, heart disease is still the number one cause of death, and there are over eight hundred thousand heart attacks a year in the United States.

Yet another parallel between statins and GLP-1s is the increasing potency of the drugs and their effect as the field moved forward. For statins, the LDL-lowering effect between the first available statins and more potent ones, like rosuvastatin (Crestor), that came along more than fifteen years later was striking. In the era of lower potency statins, there was no indication that these drugs could induce type 2 diabetes. In 2012, I wrote an op-ed in the *New York Times* that highlighted the risk of type 2 diabetes with potent statins, newly acknowledged by the FDA, of about 1 in 167 people taking Crestor 20 mg per day. While that's a low absolute risk (0.6%), the number of people taking potent statins is high. Many studies since have reinforced that there is a small but unequivocal type 2 diabetes risk for potent statins, likely due to impaired insulin sensitivity. As I've emphasized, it's imperative to avoid type 2 diabetes, if possible—we certainly don't want that to be treatment induced. The main point is that it took many years after statins were first available, and increasing their doses and potency, to recognize the risk. For the GLP-1 drugs, it will take many years to sort out whether there will be unknown, unanticipated side effects ("unknowns") for people taking increasingly potent preparations, like triple-receptor activators, for extended periods.

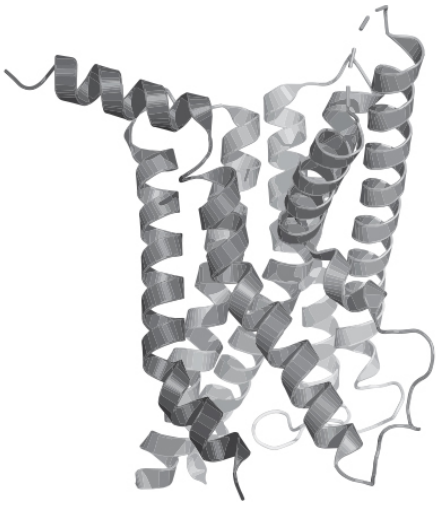
This chapter opened with the following questions: Why did we let twenty years slip before we recognized the true potential of GLP-1 drugs? What if we had AI in the early 2000s and could ask GPT-4 how to make a long half-life peptide that mimicked this naturally occurring incretin, and what would be its potential use?

The most far-reaching accomplishment in AI for life science, recognized by the 2024 Nobel Prize in Chemistry, has been the ability to predict the three-dimensional structure of the protein universe (more than two hundred million

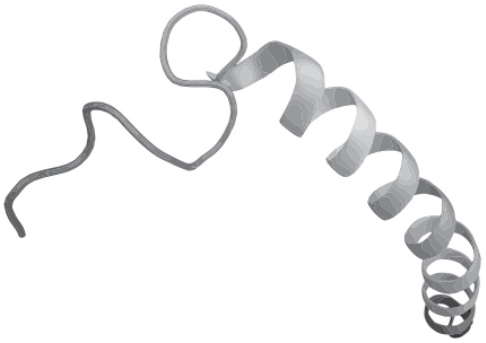
proteins) from a single, linear sequence of amino acids. This was called AlphaFold2 by a team of thirty scientists at Google DeepMind, led by Demis Hassabis and John Jumper. This required a jump from deep learning, using a convolutional neural network, to a transformer model, which corresponded to a striking improvement in predicting protein structure accuracy, from 59 to 92 percent, and from the median level of 6.6 angstroms to 1.5 angstroms (0.15 nm). The diameter of an atom is 2 to 3 angstroms, so this is predicting protein structure at the atomic level. The discovery of transformer models was made by a team at Google in 2017, distinctly different from previous deep-learning models. Instead of having to look back and forth from each word in a sentence (called a recurrent neural network), the transformer model could contextualize a whole sentence at once. Over time, with ingestion of hundreds of thousands of books, Wikipedia, and much of the content on the Internet, this gave way to large language models, such as ChatGPT and GPT-4.

What do GPT-4 and AlphaFold2 have to do with GLP-1 drugs? Figure 4.5 provides a schematic of the structure of the two prototypic GLP-1 drugs, semaglutide and tirzepatide, and the biologic gut peptides (incretins) they activate. They look... complicated. Yet the new AI models can readily predict the three-dimensional structure resulting from any changes to a GLP-1 peptide drug.

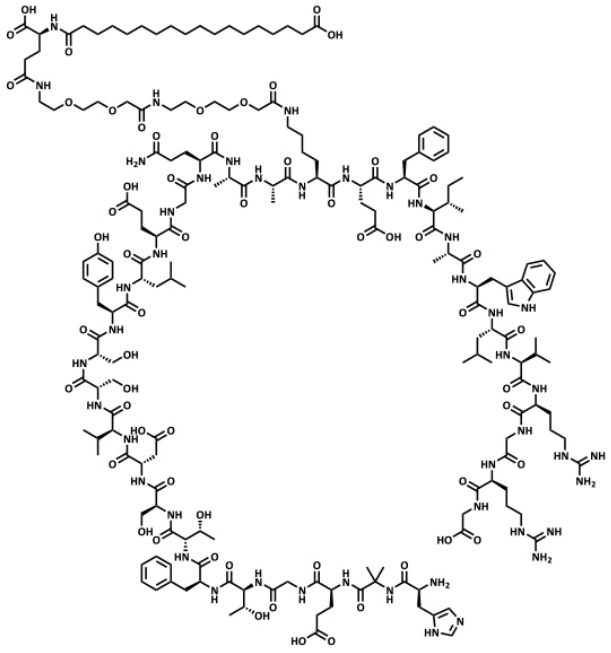
With appropriate learning from all prior GLP-1 work and related chemistry and structural biology, the transformer AI models can design new peptide structures that would be anticipated to be more potent than existing molecules. This can even extend to small molecules—pills—that haven't yet been conceived. We've already seen how AI led to a new structural class of antibiotics. The last time that occurred, it took more than three decades! This included discovery of molecules active against highly resistant bacteria, such as methicillin-resistant staphylococcus aureus. So had AI been available at the dawn of GLP-1 drugs getting approved more than twenty years ago, the path to longer half-life agents with more potent effects would likely have been much faster.



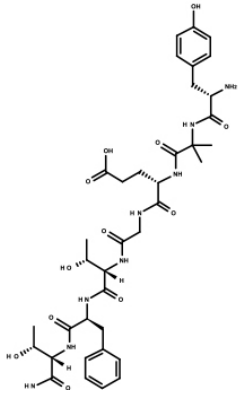
**Glucagon-like peptide-1 receptor**

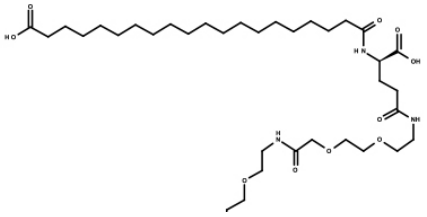


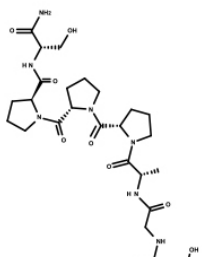
**Gastric inhibitory polypeptide**



**Semaglutide**







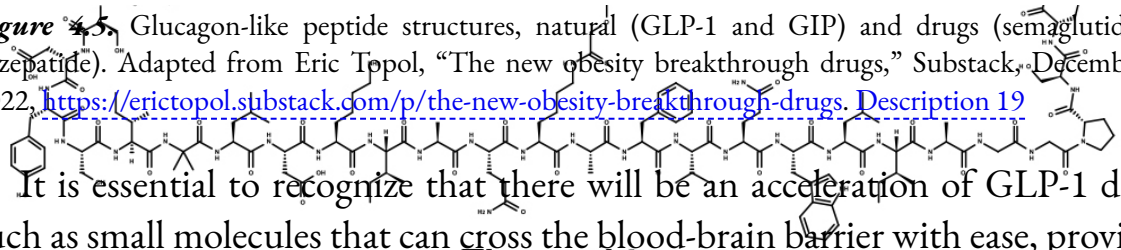



***Figure 45.*** Glucagon-like peptide structures, natural (GLP-1 and GIP) and drugs (semaglutide and tirzepatide). Adapted from Eric Topol, "The new obesity breakthrough drugs," Substack, December 10, 2022, https://erictopol.substack.com/p/the-new-obesity-breakthrough-drugs. Description 19

It is essential to recognize that there will be an acceleration of GLP-1 drugs, such as small molecules that can cross the blood-brain barrier with ease, providing stronger modulation on behaviors, or new molecules with substantially fewer gastrointestinal side effects. Like the plane that left late, AI is going to make up time in the future refining GLP-1 drugs as we know them today.

The impact of GLP-1 drugs in medicine—for improving patient outcomes—will likely be more substantial than any prior class of medications, including statins. The development of these drugs embodies a major triumph for the unmet need of high efficacy and safety for the huge population of people with obesity and type 2 diabetes. But the AI progress we have witnessed will help us with similar conquests over the greatest challenges.

We are now seeing biomedical discoveries at a breakneck pace, and they will be moving along at high velocity. Now that we have begun to crack obesity, one of the most daunting conditions, the future is bright for finding new solutions to many refractory killer diseases.

# 5

# Cardiovascular Disease

At the beginning of the twenty-first century, you might have thought cardiovascular disease was going to take a back seat to cancer as the leading cause of death. Some people projected there would be substantially less need for cardiologists. But to this day, heart disease still accounts for the greatest loss of lives of any disease, not just in the United States but globally.

The extraordinary progress made in my medical specialty is reflected in a graph that has been stuck in my mind since the first time I saw it (and didn't decide to quit cardiology). While the graph (fig. 5.1a) shows a marked reduction for heart disease over half a century, the latest US data available indicates it has been increasing steadily since 2019 (fig. 5.1b), now with about one hundred thousand more deaths per year from heart disease than cancer (approximately seven hundred thousand vs. six hundred thousand, respectively, about 17 percent more). In 2010, there were eighteen heart disease deaths per ten thousand people; in 2021, that *increased* to twenty-one per ten thousand people. In contrast, during this same period, cancer death rates *decreased* to fourteen per ten thousand people. In 2024, most Americans did not know that heart disease was the leading cause of death.

These days, there's a heart attack about every thirty-four seconds in the United States and a death attributable to these events every eighty-three seconds. Rather than the curves for fatalities from heart disease and cancer coming together, they

are diverging. And the data are age-adjusted, which should markedly reduce the potential confounding effect of an aging population.

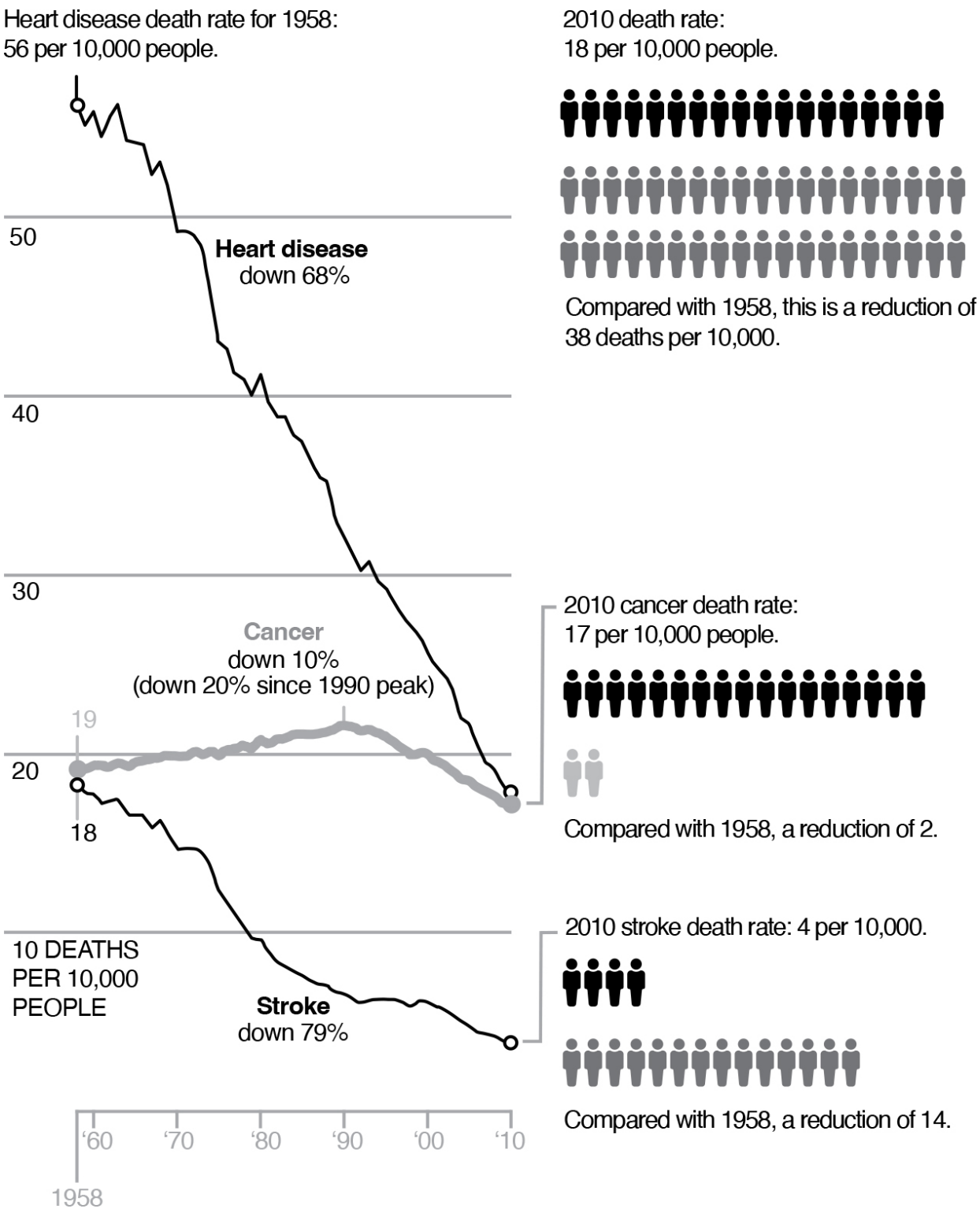



***Figure 5.1a.*** Reduction in United States deaths from heart disease, 1960 through 2010. Adapted from George Johnson, "Why everyone seems to have cancer," *New York Times*, January 4, 2014, https://www.nytimes.com/2014/01/05/sunday-review/why-everyone-seems-to-have-cancer.html?pagewanted=print. Description 20

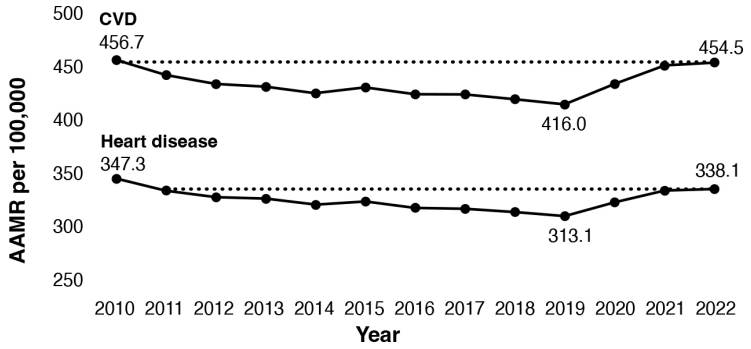



***Figure 5.1b.*** Recent increase in heart disease–related deaths, 2010–2022. AAMR: age-adjusted mortality rate. Adapted from Rebecca Woodruff et al., "Trends in cardiovascular disease mortality rates and excess deaths, 2010–2022," *American Journal of Preventive Medicine* 66, no. 4 (April 2024): 582–89, https://doi.org/10.1016/j.amepre.2023.11.009. Description 21

What accounts for the reversal of this once promising curve for heart disease? The factors that helped achieve substantially less heart disease fatalities include markedly less cigarette smoking (from 45 percent in the 1950s to about 12 percent today), the use of statins (now in approximately one in four Americans), better control of high blood pressure, and procedures to restore blood supply, whether it be in the midst of a heart attack or in nonurgent settings with bypass surgery or stenting. In addition, various improvements for treating heart failure and serious heart rhythm abnormalities have led to extension of life in these high-risk populations.

It's hard to pinpoint the reasons our hearts started killing us in greater numbers again. But we know the diabesity epidemic must be one of them. A deterioration across the many facets of lifestyle+ is likely also playing a part. Ironically, unlike other age-related major diseases, 80 to 90 percent of heart disease can be prevented by attention to the lifestyle+ factors discussed in part 1. It is indeed the most preventable of the chronic killers discussed in this part of the book.

## SCREENING FOR ATHEROSCLEROSIS

The principal basis for heart and blood vessel disease is atherosclerosis. The literal translation of that medical term is hardening of the porridge, from the Greek words *athero*, meaning gruel or porridge, and *skleros*, meaning hard. Make

oatmeal, then let the unwashed saucepan sit in the sink, and you will understand why this is bad. When the German pathologist Felix Marchand coined the term in 1904, he was referring to the fatty material with an appearance like gruel he observed inside the arteries. The fatty material is now called plaque, which forms when LDL and other lipoproteins, or molecules made of proteins and lipids that carry cholesterol, build up. The plaque accumulates over decades from progressive trapping of these lipoproteins, which is accompanied by inflammation in the wall of the artery. The atherosclerotic process can lead to different outcomes. It can be sudden, from a sudden crack or plaque rupture and blood clot, the proximal cause of a heart attack, or chronic, with the arterial pipe becoming narrowed from accumulation of plaque, limiting (but not usually completely blocking) the normal blood flow into the heart. The cumulative exposure to elevated LDL cholesterol over time and its magnitude drives atherosclerosis and is preventable with early intervention. The same process can occur in any of the arteries of the body, whether it be to the brain (cerebrovascular), the aorta with branches to the intestine and kidney, or peripheral pipes to the extremities.

The process of atherosclerosis is highly influenced by factors that damage the wall of the artery, such as cigarette smoking, uncontrolled high blood pressure, diabetes (and attendant high sugar levels in the blood), or abnormal lipids in the blood, especially LDL cholesterol. Atherosclerosis has been likened to a tumorlike disease driven by the smooth muscle cells in the artery wall. In parallel, the shared lifestyle+ risk factors and underpinning of chronic body-wide inflammation with cancer have been duly noted. There's also a component of self-directed immunity that has been increasingly documented, adding to the knowledge that people with autoimmune diseases have a considerably higher risk of developing heart disease. Even though coronary disease typically shows up in older adults, it's a lifelong process with clear-cut evidence of early plaque formation in teenagers and young adults. Some mild coronary artery plaque formation is remarkably common, some would say pervasive, as we age; by age thirty, the majority of people have at least some evidence of early coronary atherosclerotic plaque (fig. 5.2).

The sooner in life all underlying, driving lifestyle+ factors are addressed, the less chance there will be of significant accumulation of plaque or plaque ruptures. What can't be emphasized enough about the major age-related diseases—heart, brain, and cancer—is that they typically are incubating for about twenty years or more before they declare themselves clinically. What an opportunity that represents to prevent their occurrence.

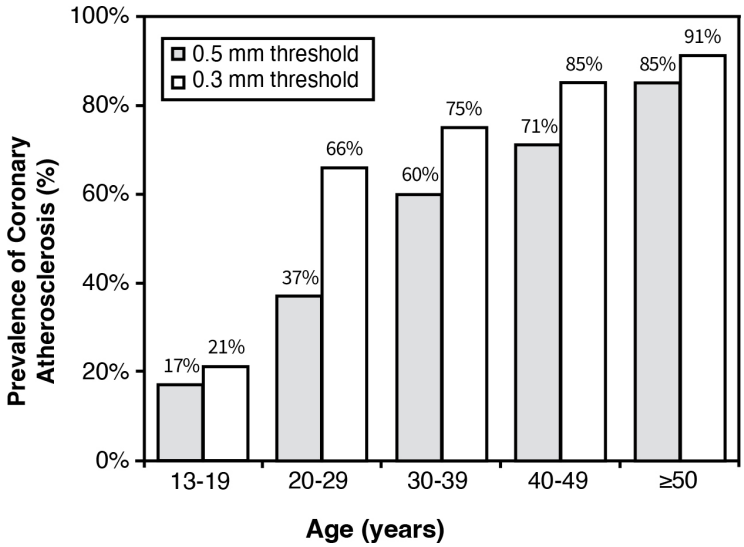
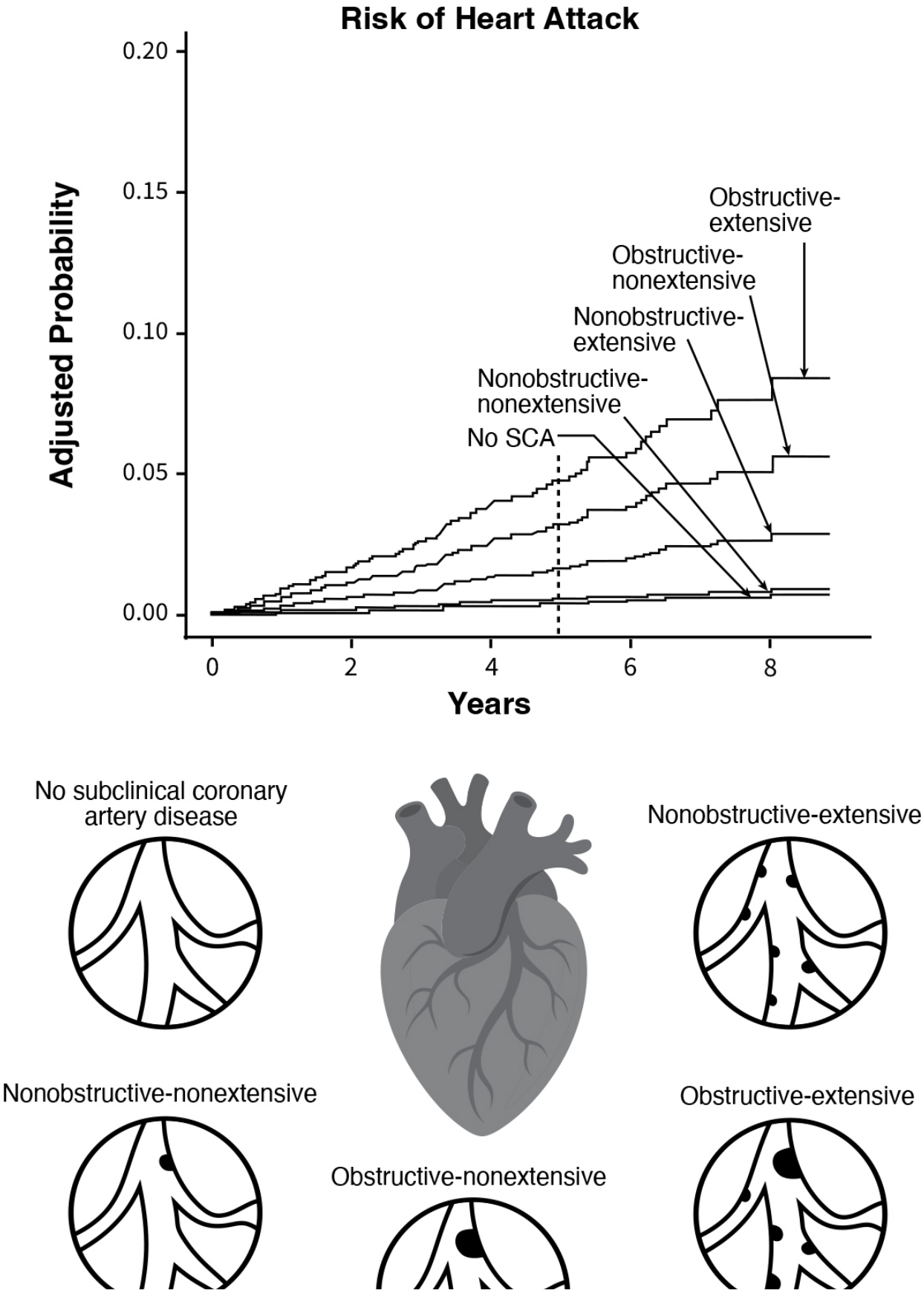



***Figure 5.2.*** Prevalence of coronary atherosclerosis seen at young ages, with different thresholds of lesion size. Adapted from EM Tuzcu et al., "High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: Evidence from intravascular ultrasound," *Circulation* 103, no. 22 (June 2001): 2705–10, https://doi.org/10.1161/01.cir.103.22.2705. Description 22

An important prospective study in Denmark used CT (computed tomography) coronary angiograms to assess more than ninety-five hundred people aged forty years or older *without symptoms* or known heart disease and followed them for three and a half years. About half of the participants had no evidence of atherosclerosis. The rest fit into one of the categories in figure 5.3. Obstructive or extensive coronary artery disease was associated with an eight- to ninefold increased risk of heart attack; when both were present, the risk increased to thirteenfold, compared with those without atherosclerosis. The results for heightened risk of death or heart attack were similar. Even nonobstructive but extensive atherosclerosis carried a threefold risk of a heart attack. These findings emphasize the high frequency of coronary artery disease in people without any symptoms. Even with short duration of follow-up, nonobstructive disease carries

significant risk. That's why screening is so important. Wouldn't it be great to know who is at high risk without doing CT coronary angiograms indiscriminately for the population?

The American Heart Association calls healthy diet, physical activity, avoidance of nicotine, healthy sleep, healthy weight, and healthy levels of lipids, blood glucose, and blood pressure "Life's Essential 8." These factors are key drivers of atherosclerosis, but genetics also plays a role. While not common, that's why some people who are perfectly healthy, with no hypertension, diabetes, or lipid disorder, can go on to have heart attacks or require procedures to restore normal blood supply. About one in twenty people with coronary atherosclerotic disease present before age forty to forty-five, known as premature CAD, and up to 60 percent without any history in their family. As discussed in chapter 1, polygenic risk scores can help determine a person's risk of heart disease. They are being implemented in some health systems in the United States, such as Mass General Brigham, and in some life insurance companies. They are also increasingly becoming available through several commercial entities including 23andMe, Genetype, Genomic PLC, Open DNA, Ambry Genetics, Myriad Genetics, and Haplotype Labs. For the individuals from Mass General Brigham who scored in the top 10 percent, classified as high risk, there was about a twofold risk of developing heart disease across ancestries, up to nearly a fourfold increase among people of Asian ancestry. In a large NIH-supported project that defined high risk for coronary disease as scoring in the top 5 percent, that twofold risk across ancestries was independently replicated. In twenty-five hundred people assessed for ten different disease polygenic risk scores, coronary heart disease was the one that detected high risk most commonly. A prospective study of more than ninety-six thousand participants in China highlighted the importance of the polygenic risk score for predicting cardiovascular diseases, along with lifestyle, and their additive impact.


Risk of Heart Attack
Adjusted Probability
0.20
0.15
0.10
0.05
0.00
0
2
4
6
8
Years
Obstructive-extensive
Obstructive-nonextensive
Nonobstructive-extensive
Nonobstructive-nonextensive
No SCA
No subclinical coronary artery disease
Nonobstructive-extensive
Nonobstructive-nonextensive
Obstructive-extensive
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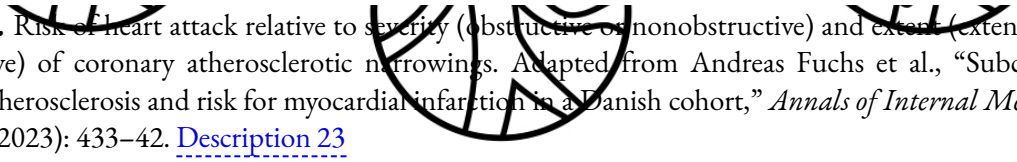


***Figure 5.3.*** Risk of heart attack relative to severity (obstructive or nonobstructive) and extent (extensive or nonextensive) of coronary atherosclerotic narrowings. Adapted from Andreas Fuchs et al., "Subclinical coronary atherosclerosis and risk for myocardial infarction in a Danish cohort," *Annals of Internal Medicine* 176, no. 4 (2023): 433–42. Description 23

Ideally, everyone would know at an early age their risk score because it's independent of (and additive to) family history of heart disease and the other known risk factors. A high polygenic risk score predicts benefit for the use of statins for lowering the risk of coronary atherosclerotic disease, a step toward primary prevention, especially when initiated at a young age. Besides guiding use of statins, knowing that one's coronary artery disease polygenic risk score puts one at high risk helps change people's behavior. I've used it in my clinic for nearly a decade to help decide whether a statin should be initiated in people with minimal or no risk factors. Unfortunately, that hasn't yet become a routine part of medical practice.

The proteins in our blood are helpful indicators for risk of heart disease. The presence of specific proteins and their levels are the basis of a proteomic score. In a UK Biobank study of twenty-two thousand participants, a proteomic score, based on over three hundred proteins, was associated with cardiovascular (and multisystem) risk, additive to the polygenic risk score, and was modifiable by exercise training. There is also a heart organ proteomic clock, based on about 850 proteins, validated in multiple population cohorts. About 2 percent of people exhibit accelerated aging of their heart, which could potentially be adapted clinically for detecting cardiovascular risk. An imminent heart attack within six months was predicted with increased accuracy when levels of forty-eight proteins and forty-three metabolites were added to clinical features. As we age, blood stem cells develop clones of particular mutations, called clonal hematopoiesis of indeterminate potential. But it's not so indeterminate after all, since CHIP is strongly associated with heart disease and clotting event risk, and could easily be incorporated in a comprehensive risk assessment. In people aged seventy years and older with CHIP, there is a sixfold increased risk of heart disease. None of these gene or protein scores have been used in clinical practice.

Even further from the clinic is the gut microbiome score for risk of heart disease. When this was added to clinical risk factors and the genomics (polygenic

risk) data, in about five thousand people followed for eighteen years, it added risk information comparable to blood pressure, cholesterol or triglyceride levels, and enhanced prediction or risk. Separately, a gut microbiome analysis of fourteen hundred participants revealed specific bacteria species that metabolize cholesterol and lower its blood level, further implicating the gut microbiome's role in modulating heart disease. What is even more striking is to see how a gut microbiome "metabolic age," as determined by specific microbial species (such as low *Prevotella*), counters the risk of cardiovascular disease in older adults. A study of the gut microbiome in nearly fifty-seven thousand participants in thirty-two countries found the prevalence of *Blastocystis*, a single-celled organism, to be associated with better cardiometabolic profiles and outcomes and less obesity, and it increased in response to a healthy diet. These studies support the notion that we may be able to modulate the risk of age-related disease by manipulating the gut microbiome.

In much more common use is a calcium score, as obtained from a CT scan. This test assesses buildup of calcium in the coronary arteries, which may or may not relate to the accumulation of atherosclerotic plaque inside the arteries. It's therefore an indirect test that is heavily marketed, such as a gift for Valentine's Day, and overused by doctors for people with no risk factors. Many patients that have been referred to me have a score above one hundred and are panicked that they are at risk from a fatal heart attack. While there is some correlation between the score, which is indexed to age, and risk of a heart attack, it applies only for scores well above three hundred. Surprisingly, I have seen a substantial number of patients with a score of zero or extremely low who have had repeat testing every year or two, which is completely unnecessary. These tests are usually not covered by insurance and typically cost $150 but can range up to $400. Nearly forty years after the calcium score tests were introduced, there are finally randomized trials underway to determine whether the results have a meaningful impact on patient outcomes. CT heart scans with contrast dye to delineate the arteries may turn out to have a role for detecting inflammation in heart arteries, which we'll get to shortly.

## DRUG TREATMENTS

Well before there were polygenic risk or calcium scores for coronary atherosclerotic disease, LDL cholesterol was fully validated as a critical biomarker, one of the most extensively studied lab tests in all of medicine. There have been numerous large clinical trials testing statins and determining the relationship between LDL level achieved and coronary heart disease (CHD) events, as shown in figure 5.4, with each data point corresponding to the acronym name of the clinical trial.

Over recent years, the safety and benefit of "the race to the bottom" of ultra-low LDL levels, well below 50 mg/dL, has been advocated, made possible by a variety of drugs: more potent statins and conjunctive use of ezetimibe (Zetia), bempedoic acid (Nexlotol), and the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (injectables, which include alirocumab [Praluent], evolocumab [Repatha], and inclisiran [Leqvio]). Notably, inclisiran is a small interfering double-stranded RNA drug that only requires an infusion every six months. The other PCSK9 blocking injectable drugs are monoclonal antibodies given once or twice a month.

It isn't clear what is the optimal target level, but in people who have manifested coronary atherosclerotic disease, it would be reasonable to get below 50 mg/dL if the drug(s) is/are well tolerated. Some physicians have extrapolated from the data accrued that going ultra-low, as close to zero as possible, even in people with elevated LDL but otherwise healthy, without risk factors, should expand health span. There's no hard evidence for that yet, but the trend in recent years has been more aggressive lowering and starting at as early an age as possible in people with elevated LDL and other cardiovascular risk factors. The results from further analysis of a recent trial in people with stable coronary artery disease indicated better protection for reduction of cardiovascular death, heart attack (MI), and stroke with LDL cholesterol lowered less than 20 mg/dL and reinforce this aggressive goal as a secondary prevention (fig. 5.5). I want to remind you of the increased risk of type 2 diabetes with the use of aggressive statin dosing, such as was seen in a randomized trial of rosuvastatin versus atorvastatin and an

overview of all trials. On the other hand, there is evidence to support the benefit of statins and low risk in people aged eighty-five years and older.

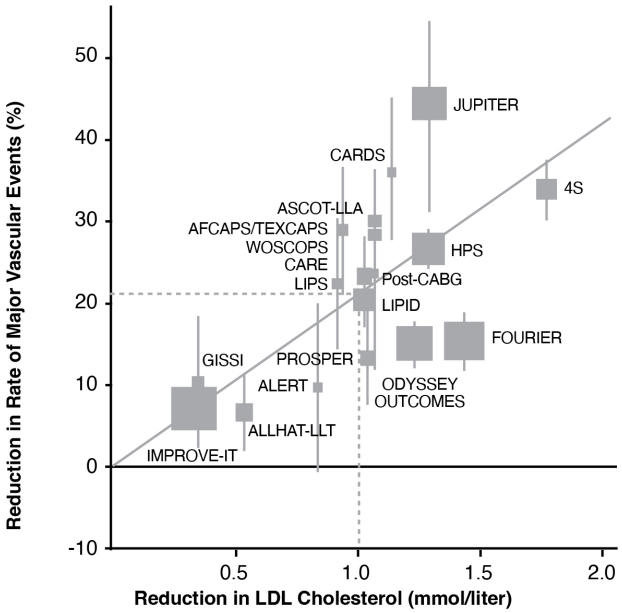



***Figure 5.4.*** Lowering of LDL cholesterol in various randomized trials and reduction of major cardiovascular events. Trial names are acronyms. Adapted from FB Mensink et al., "Pharmaco-invasive therapy: Early implementation of statins and proprotein convertase subtilisin/kexin type 9 inhibitors after acute coronary syndrome," *Frontiers in Cardiovascular Medicine* 9 (December 2022): 1061346, https://doi.org/10.3389/fcvm.2022.1061346. Description 24

There are other lipid assays, which are lab tests to find and measure a specific substance, associated with atherosclerosis. In the typical panel, triglycerides and HDL cholesterol are incorporated. As reviewed in the metabolic syndrome (chapter 1), high fasting triglycerides and low HDL are two of the main indicators of potential insulin resistance. There is a direct correlation between triglycerides and heightened risk. Unless extremely high, moderately elevated levels are usually responsive to weight loss and attention to the lifestyle+ factors. For people with high risk of heart disease and lack of response from changes in lifestyle, treatment with icosapent ethyl (Vascepa), a modified form of the fish oil called eicosapentanoic acid, may be useful. That treatment is backed up by a large randomized trial for protection against cardiovascular events, but the results remain controversial since other trials of fish oils have not been found to reduce adverse events. Olezarsen, an antisense drug being developed for high triglycerides, has been shown to be effective for a 50 percent reduction via a

monthly injection. Marked lowering of triglycerides is now being achieved with RNA-interfering drugs directed against the angiopoietin-like 3 (ANPTL3) gene and the apoprotein C3 (APOC3) gene. People with two copies of the genes for familial hypercholesterolemia are at extremely high risk for atherosclerosis at a young age. That's why it's good that a monoclonal antibody to ANGPTL3, Evinacumab (Evkeeza), has been approved for the treatment of this rare disease in children.

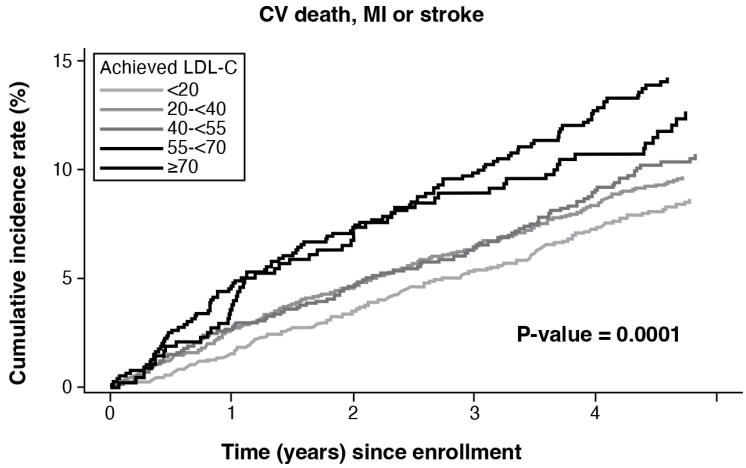



***Figure 5.5.*** Absolute level of LDL cholesterol achieved and reduction of the triad of cardiovascular death, heart attack (MI), and stroke. Adapted from Prakriti Gaba et al., "Association between achieved low-density lipoprotein cholesterol levels and long-term cardiovascular and safety outcomes: An analysis of fourier-ole," *Circulation* 147, no. 16 (April 2023): 1192–203, https://doi.org/10.1161/CIRCULATIONAHA.122.063399. Description 25

The ANGPTL3 directed (zodasiran) and APOC3 targeted drugs (plozasiran) are given once every three months, have a broad beneficial effect for multiple lipids, and may prove to be especially useful in people with mixed lipid abnormalities.

The relationship between HDL, often referred to as the "good cholesterol," and cardiovascular outcomes is much different from LDL and triglycerides. HDL cholesterol is generally regarded as a bystander, not a driver of atherosclerosis. Paradoxically, very high levels of HDL, above 80 mg/dL, may carry a higher risk of all-cause or cardiovascular death. Not so "good" after all, these high levels are often associated with dysfunctional HDLs that don't act in their scavenger role of cholesterol as expected. Like high triglyceride management, low HDL levels (<40 mg/dL in men, <50 mg/dL in women) typically respond to the same

lifestyle alterations. In contrast to the large body of evidence of lowering LDL cholesterol, active efforts for modifying triglycerides or HDL cholesterol from clinical trials have less correlation or proof of improving cardiovascular outcomes. Still, what your doctor calls good lipid panel results *are* good.

Another vexing lipoprotein that has long been without a treatment, but with clear-cut risk, is lipoprotein(a) or Lp(a). Less than 0.5 percent of people undergo Lp(a) testing even though high levels constitute a risk factor for heart disease. Recently, a pill has been demonstrated to lower Lp(a) by 65 percent, but this is in early human trials, and, although likely, we don't know for certain it will lower the risk of coronary atherosclerotic disease. There are also several ongoing efforts for small molecule drugs and biologics (small interfering RNAs) that are targeting Lp(a). It would not be surprising that if one of the drugs to lower Lp(a) in the future works, then its assay will be added to one's routine lipid panel.

Many other lipid markers could be assessed that aren't currently, but one that should be is Apolipoprotein B (apoB). While it typically correlates with LDL cholesterol, many impressive studies indicate it is a more accurate predictor of risk. About 20 percent of people who have a normal LDL cholesterol will have a high apoB, denoting risk of cardiovascular disease. That makes sense, because it is a more direct measurement—it is apoB that carries cholesterol in our blood. This protein encapsulates LDL particles but also intermediate-density lipoproteins (IDL), very low-density lipoproteins (VLDL), and Lp(a). Some people have a high apoB level but normal LDL cholesterol and are at high cardiovascular risk, which is why it's an important lab test for people at risk to get, such as with metabolic syndrome, high triglycerides, or high polygenic risk score. It should be in the standard lipid panel, covered by insurance, but it usually isn't. It's worth getting at least once, or on occasion with routine lipid panels, to see if there is a divergence between one's LDL cholesterol and apoB levels, and help monitor one's level of risk, such as needing to increase a dose or potency of a statin medication. Perhaps someday its glaring omission, defying such a strong evidence base, will be fixed, and apoB will be part of standard panels. In the meantime, a proxy for apoB is to determine the non-HDL cholesterol by calculating total cholesterol minus HDL cholesterol. While not as good as a direct apoB

measurement, that can usually be derived from routine lipid panels and correlates rather well.

The good news is that there is a surge of new drugs for abnormal lipids, mostly biologic small-interfering RNA, targeting genes that were formerly not possible. This completely changes the landscape of both screening and treatment options for patients deemed high risk. We also have good alternatives for patients who cannot tolerate statins, such as bempedoic acid, and multiple PCSK9 blockers.

While managing abnormal lipids is a mainstay to prevent atherosclerosis, tight control of hypertension and diabetes, when present, is equally important. These risk factors are connected, and blood supply to all organs of the body relies on our cardiovascular health. Beyond that direct connection from the heart and blood vessels to all our organ systems, there is considerable overlap of the risk factors that affect each of them.

With the preponderance of evidence that inflammation is a key culprit underpinning atherosclerosis, and that high-sensitivity C-reactive protein (hs-CRP) is a blood biomarker for body-wide inflammation, the stage was set for clinical trials—to test the hypothesis that a potent anti-inflammatory agent could suppress heart disease. Published in 2017, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) enrolled over ten thousand participants who had a prior heart attack, an elevated hs-CRP, and were randomly assigned to three different doses of the drug (canakinumab, a monoclonal antibody to interleukin -1β), given every three months by injection, or a matching placebo for nearly four years. The drug reduced hs-CRP by 40 percent at the higher doses, which also achieved, without any effect on lipid levels, a significant reduction of heart attack, stroke, and cardiovascular death. However, there was a small (affecting about twelve per ten thousand individuals treated) but significant increase in fatal infections or sepsis for the drug (0.3%) compared with placebo (0.18%), which reflects its immune suppression effect. Something else quite noteworthy about the outcomes of this trial: a significant reduction of fatal cancer and lung cancer. We can conclude that a potent anti-inflammatory drug suppressed both heart events and cancer, with a small absolute increased risk

of fatal infection. The drug manufacturer, Novartis, decided not to pursue the indication for cardiovascular event reduction.

Another pass at this question was with colchicine, undertaken a few years later. As opposed to the monoclonal antibody, this potent anti-inflammatory, discovered over three thousand years ago and isolated in the 1800s, is inexpensive and administered orally. Its main side effects are gastrointestinal, particularly diarrhea in about one in four people. A randomized trial in nearly five thousand patients within thirty days of a heart attack, followed for two years, showed the colchicine assigned group to have a significant reduction of cardiovascular events compared with placebo (absolute benefit in about two per one hundred individuals). Like the CANTOS trials, there was an issue with infections—namely, an increased incidence of pneumonia, seen more frequently with colchicine.

Then came another colchicine large randomized trial of over five thousand people with chronic contrary artery disease with a 30 percent reduction in cardiovascular events (nearly three per one hundred people benefit), but there was an excess of noncardiovascular deaths (about two per one thousand people). Collectively, the consistent results of these three trials make a strong case that an anti-inflammatory drug can reduce heart attacks, stroke, and cardiovascular deaths. In June 2023, the FDA approved a version of colchicine (0.5 mg), called Lodoco, as the first drug ever to treat cardiovascular inflammation. It has a retail price at least tenfold higher than the generic version (0.6 mg) that can be prescribed by doctors. But neither is used in routine medical practice, even though the aggregate data, as summarized in figure 5.6, based on a meta-analysis of five randomized trials, is quite supportive. When the trials were pooled, there was no significant risk of infection or pneumonia. In 2024, an additional sixth randomized trial in patients who'd had a stroke further confirmed these benefits for colchicine.

It turns out there's another way to get at coronary artery inflammation. A CT scan coronary angiogram can detect the level of inflammation from the fat surrounding each of the three coronary arteries. When that was done in over forty thousand people, with follow-up in a subset to nearly eight years, more than 80 percent of individuals did not have obstructive coronary disease (no significant

atherosclerotic narrowing). But there was a marked increased risk of major adverse outcomes in this group, accounting for more than 60 percent of the major adverse cardiovascular events. In the cohort with extended follow-up, there was a striking relationship of arterial inflammation and such major adverse events. For example, there was a thirtyfold risk of cardiovascular death for inflammation detected in all three arteries, and a thirteenfold risk for one artery inflamed, as compared to none, over the extended follow-up (fig. 5.7). These findings were distinct from and additive to the traditional clinical risk factor determination.

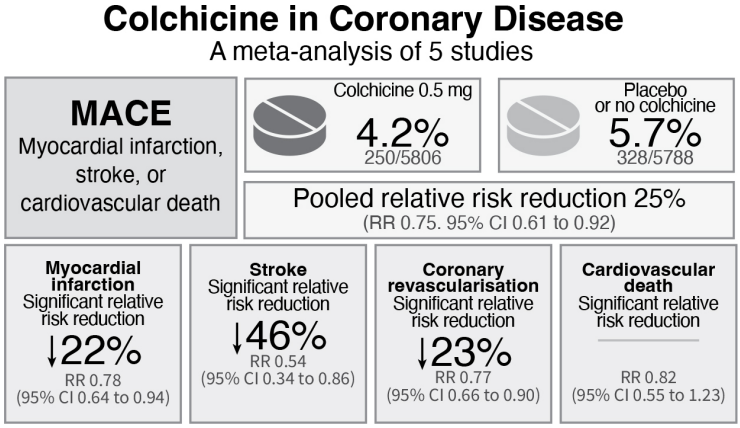



***Figure 5.6.*** Summary of results for five randomized trials of colchicine for coronary artery disease. Adapted from Aernoud Fiolet et al., "Efficacy and safety of low-dose colchicine in patients with coronary disease: A systematic review and meta-analysis of randomized trials," *European Heart Journal* 42, no. 28 (July 2021): 2765–75, https://doi.org/10.1093/eurheartj/ehab115. Description 26

There's also the ability to determine high-risk, vulnerable plaque (without significant narrowing or limiting of blood flow) more directly by imaging inside the coronary arteries. A randomized trial of sixteen hundred patients with these vulnerable plaques compared intervention (with a balloon angioplasty or stenting) versus medical therapy and found a substantial reduction of major cardiac events during two years of follow-up. These results have challenged dogma that stenting should only be done for significant flow-limiting, arterial narrowing, often colloquially referred to as "blockages."

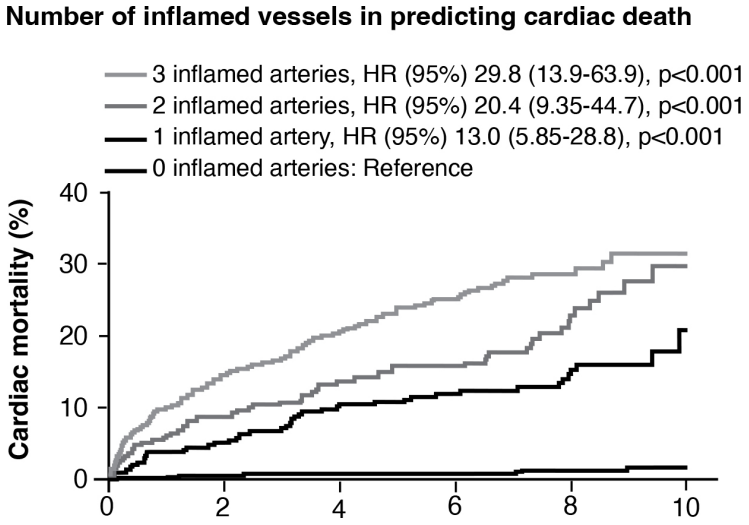



***Figure 5.7.*** Relationship of inflamed arteries (by AI of CT scan) and heart-related death. Adapted from Kenneth Chan et al., "Inflammatory risk and cardiovascular events in patients without obstructive coronary artery disease: The ORFAN multicentre, longitudinal cohort study," *Lancet* 403, no. 10444 (June 2024): 2606–18, https://doi.org/10.1016/s0140-6736(24)00596-8. Description 27

From a synthesis of all these trials, we can acknowledge that body-wide or heart artery inflammation is a risk factor for major adverse cardiovascular events, and that anti-inflammatory drugs can improve outcomes. Yet, the cardiovascular community has not moved on to change practice, acknowledging our ability to detect inflammation and to do something about it. Perhaps that will eventually sink in when we have better anti-inflammatory drugs.

## FORECASTING AND PREVENTION

The longtime window it takes for atherosclerosis to build up provides an opportunity to determine who is at high risk for developing coronary artery disease, closely follow these individuals, and intervene early to modify any risk factors, which now, as we just reviewed, include inflammation (fig. 5.8). Assessment of an individual for risk should now go well beyond the traditional clinical factors of diabetes, obesity, smoking, lack of physical activity, and high LDL cholesterol.

The insights derived from these heart inflammation reports helps add another way to forecast a person's entanglement with heart disease. Besides the well-

established clinical risk factors, there are many other results that can help pinpoint high-risk individuals. The list includes polygenic, gut microbiome, and proteomic risk scores; heart aging clock; CHIP; inflammation biomarkers such as hs-CRP, along with AI interpretation of the retina photo, and if available, chest X-ray. The retina and chest X-ray are examples of what has been called "opportunistic medical imaging," since getting a handle on heart disease risk isn't the reason why the tests were ordered. But with AI, the heart artery calcium score can be accurately determined from the retina or the chest X-ray. The retina photo can also be used to predict the risk of heart attack and stroke. Epigenetic age (DNA methylation) clocks that indicate accelerated body-wide aging are correlated with increased risk of adverse cardiovascular outcomes.

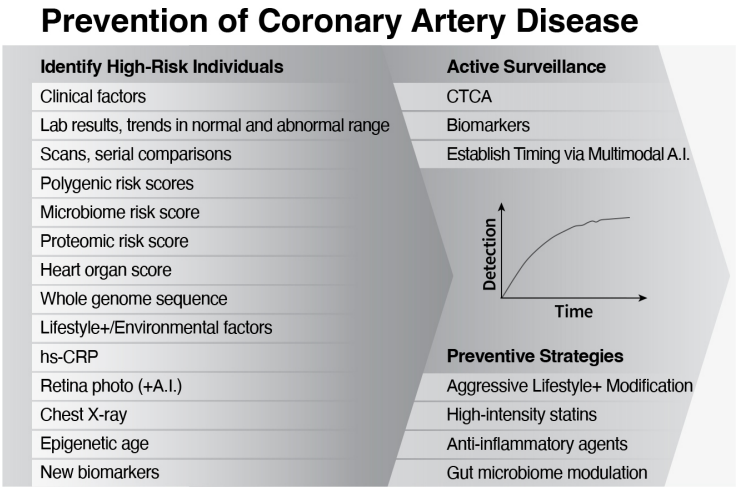



***Figure 5.8.*** Prevention of coronary artery disease. A multimodal AI approach for identifying high-risk individuals, putting them on active surveillance, and aggressive steps to block the progression of atherosclerosis and inflammation. Description 28

Once a person is deemed at high risk from this assessment, comprehensive lifestyle+ factors can be promoted and close monitoring can be initiated with a CT scan for coronary artery inflammation, blood markers for body-wide inflammation. More aggressive blood pressure management, targeting less than 120 mm Hg, has been shown to prevent major vascular events in large randomized trials. High-intensity statin and, if unresponsive, anti-inflammatory drugs such as colchicine can potentially be used, especially if there are persistent elevated markers of inflammation, despite aggressive efforts to improve lifestyle. Better anti-inflammatory drugs or gut microbiome modulation will undoubtedly

be available in the future. Such a heart disease forecasting effort reflects the primacy of inflammation as a driver, along with the opportunity to suppress it during the long process that it takes for atherosclerosis to declare itself. We're not doing this yet, but it's the path to prevention or delay of a major age-related disease.

## ATRIAL FIBRILLATION

There's another heart condition that is age related, common, more heritable, and more preventable than generally acknowledged. Atrial fibrillation is the heart rhythm abnormality that is the leading cause of embolic stroke, with a fivefold increased risk. It affects more than sixty million people worldwide, and more than five million in the United States. Age is the most important risk factor. The risk increases sharply from 0.2 percent for people in their thirties to 4 percent in their seventies. The lifetime risk of this heart rhythm abnormality is nearly 25 percent for adults over the age of forty-five. Besides stroke, AF carries an increased risk for heart failure and mortality. There is expected average loss of 9 life years for people less than or equal to age fifty, and 5.4 years for those age seventy at the time of diagnosis.

Many of the risk factors for this condition overlap with atherosclerotic cardiovascular disease, including age, obesity, hypertension, diabetes, smoking, lack of physical activity, and air pollution. But some risk factors are more specific to atrial fibrillation, including sleep apnea or disorder, thyroid disease, kidney disease, chronic obstructive pulmonary disease, mental health disorders, high intake of sugary beverages, high testosterone in older men, increased height, young elite athletes, extreme endurance exercise, and gum disease. A common misconception is that caffeine intake is associated with atrial fibrillation risk; it is not. If anything, from all the studies available, it is linked to a lower risk. An important, if not surprising, randomized trial showed that weight loss in overweight and obese individuals reduced the recurrence and symptom burden of this particular heart problem.

Like coronary artery disease, atrial fibrillation is largely asymptomatic, and about 10 to 20 percent is undiagnosed. However, the ease of detection has been

markedly enhanced with AI algorithms embedded in some smartwatches and fingertip sensors that can be used to record one lead of the electrocardiogram when a person feels they may have an abnormal heart rhythm.

We can't change our age or height or genetics. But we can determine our genetic risk with a polygenic risk score. Over one hundred genetic loci have been identified to associate with risk of developing atrial fibrillation. The Mass General Brigham study found over 8 percent of their patient population assessed had a high polygenic risk score, with a doubling of risk across white, Black, and Asian ancestries. In a large study of twenty-five thousand adults, high polygenic risk in the top 3 percent was associated with a doubling of the odds ratio for multiple ancestries. A polygenic risk score can also help predict the risk of stroke associated with atrial fibrillation. Reflecting the higher risk noted with gum disease, inflammation blood markers such as hs-CRP, interleukin-6, and tumor necrosis factor are associated with increased risk, as is brain natriuretic peptide. The heart organ proteomic aging clock reviewed earlier also is predictive. Not unexpectedly, with their anti-inflammatory and marked weight loss effects, the GLP-1 drugs tested in multiple randomized trials have shown substantial reduction of atrial fibrillation. And finally, epigenetic markers by DNA methylation can help identify people at increased risk for atrial fibrillation. But the sad fact is that none of these omic tests are used in mainstream clinical practice.

Using AI, the 12-lead electrocardiogram in people who have never had this kind of heart arrhythmia can be used to help accurately gauge risk of subsequently developing AF and stroke. Blood thinners for people with atrial fibrillation at increased risk for stroke are highly effective.

A common thread runs through these two major age-related cardiovascular conditions that pose serious risks of heart attack, stroke, and death. The conditions are both often subclinical—a large proportion of the population are incubating the disease or are sitting at high risk. Using genetic and inflammatory markers, we can do far better at detecting who they are and then taking steps to prevent these conditions from developing. In short, we know how to suppress the most important age-related cardiovascular diseases now—expand health span—but we're not doing it.

Let's turn to cancer. We'll see many parallels.

# 6

# Cancer

We're stuck in the 1960s in our approach to cancer. By 2050, the number of people dying from cancer is projected to nearly double, but we have not yet incorporated over half a century of extraordinary breakthroughs in the understanding and use of certain biologic mechanisms that can stop cancer from killing us now. No less the implementation of comprehensive prevention strategies such as banning tobacco. We can define risk at the individual level, make the diagnosis very early and more accurately, and then treat patients far more safely and effectively. Since nearly half of us will develop a form of cancer during our lifetime, we can't forge a major health span expansion without getting our arms around it.

For far too long, we've waged a "war" on cancer (presidentially declared in 1971), with too many people losing the battle against it. But enough with the war metaphors. This isn't a brute force military operation. Health span expansion requires smart use of our cancer knowledge base with the five dimensions of lifestyle, omics, AI, cells, and drugs/vaccines. We know so much about cancer now that we can transform the practice of medicine, and much of that knowledge is ready for you to use.

To get our arms around it, let's look first at where our understanding of cancer's biology stands. How does a cancer get started? There are three major theories, which are not mutually exclusive. The basic notion of the somatic

mutation theory is that a gene (an "oncogene") acquires mutations that either activate it or cause it to lose its ability to suppress tumors. As an individual ages, more and more cells die due to instability in the genome, exhaustion of stem cells, dysfunction of mitochondria, and impaired disposal of misfolded proteins and senescent cells. Intriguingly, these processes are the same as the hallmarks of cancer. In our genome of three billion letters, several thousand mistakes occur each time a cell divides. As we age, mutations accumulate in our cells at a rate of fifteen to fifty per cell each year. While most of these don't give rise to cancer, scientists have discovered over five hundred genes that are more likely to cause cancer when mutations arise in their DNA sequences. These are known as driver genes, because when they are mutated, they drive cell growth and can induce clones of cancer cells to kick off the disease.

In contrast to this cell-based origin theory is one that is tissue based. It occurs locally, where there is some carcinogenic exposure, disruption of tissue, and abnormal immune response. The third theory is tersely known as "bad luck" attributed to a random mistake in the process of stem cell replication. Given the frequency and diversity of cancer, it wouldn't be at all surprising that all these mechanisms result in cancer one way or another.

The field of spatial biology has transformed our understanding for how cancer evolves (fig. 6.1). Through sequencing of hundreds of thousands, if not millions, of single cells of cancer tissue over time (including their DNA, RNA, proteins, metabolites, and methylation pattern), we are learning precisely how a tumor grows over time and in space. Analysis of these massive datasets requires the use of AI tools and new algorithms. This exquisite spatiotemporal characterization, which can be displayed in 3D, maps out the clones and subclones of cancer cells. It also depicts the new proteins that are expressed on their surface, known as neoantigens. We've learned so much about the importance of the tumor microenvironment, the noncancerous ecosystem surrounding the tumor that consists of immune cells, blood vessels, and a large network of proteins and other molecules called the extracellular matrix. The spatial biology 3D maps the tumor microenvironment and the metastatic seeding to other organs.

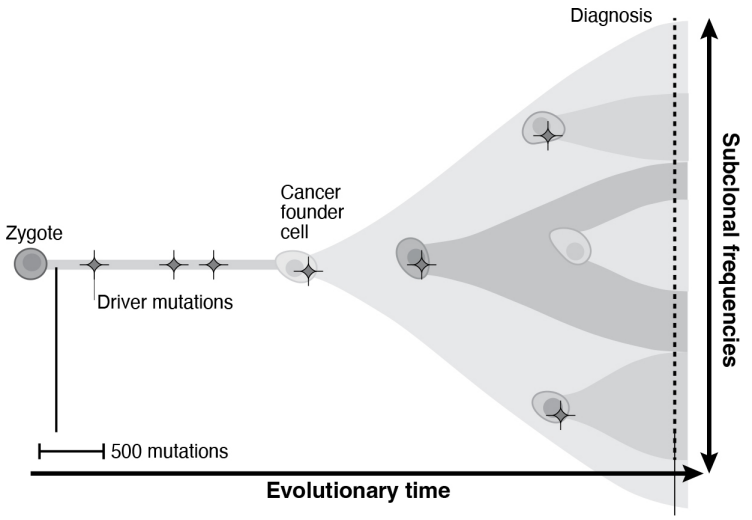



***Figure 6.1.*** Evolution of cancer. Accumulation of mutations in cells over time leading to a founder cancer cell with a driver mutation and different clones of tumor before the diagnosis is made. Adapted from Zaira Seferbekova et al., "Spatial biology of cancer evolution," *Nature Reviews Genetics* 24, no. 5 (May 2023): 295–313, https://doi.org/10.1038/s41576-022-00553-x. Description 29

When reviewing a slide of a tumor biopsy, we used to just say a person's cancer tissue was heterogeneous—that is, not uniform. But now spatial biology goes much deeper in its analysis to reveal the evolutionary background landscape of the tissue—a sort of cellular time machine. Cells can be barcoded to track their fate, sequencing their mitochondrial DNA, the relative abundance of tumor clones over time in different parts of the body, and differentiate nonmalignant processes, such as clonal proliferation of blood stem cells (hematopoiesis). It had long been recognized that approximately 25 percent of cancers are preceded by chronic inflammation at the site of the tumor origin, but we recently learned that a type of intestinal T cell is capable of initiating cancer on its own. Notably, spatial mapping of the pancreas in healthy adults demonstrated *hundreds* of small cancer precursor lesions called PanINs (short for pancreatic intraepithelial neoplasia), almost all of which contain KRAS (the leading driver gene of pancreatic cancer) hot spot mutations. Given how many PanINs people have, it's amazing more cancers don't develop in us. Our bodies have strong defenses.

Alongside high-tech mapping, treating patients has enlightened us. A woman who developed breast cancer at the age of fifty-one had precursor clones of cancer evident at thirteen. Another woman diagnosed at fifty-three had cancerous clones at seventeen. A gap of more than ten years was found from the appearance of

driver mutations to founder cancer cells, another substantial lag to diagnosis. This is a long road to cancer, on which we can detect its occurrence at a precancerous state, and certainly before it has spread from its site of origin to other parts of the body.

This is a profoundly powerful insight. It's a key to unlocking tools we can use against *all* the major age-related diseases, providing opportunities to change their natural history. We can prevent these diseases years before they become full blown.

It's unusual for someone to die from cancer unless it has undergone metastasis, spreading from one part of the body to another. Metastasis is what kills more than 90 percent of those who die from cancer, with many different proximal causes of death, particularly organ failures, not necessarily related to the body's tumor burden. Metastasis-initiating cells acquire aggressive features that enable invasive migration via blood vessels or lymphatics in various ways, including evasion of the immune system, epigenetics, and metabolic adaptation. Sampling blood for circulating tumor cells or cell-free tumor DNA can track this migration, which we've learned can be quite aggressive while we sleep. In a study with samples from individuals with breast cancer taken at four o'clock and ten o'clock in the morning, 78 percent of circulating tumor cells were found during sleep. While there's certainly no practical strategy for avoiding sleep to reduce metastasis, this study points to how aggressive cancer may be in exploiting our immune system.

It is remarkable just how opportunistic cancer cells can be. For cancer cells circulating in the blood, just two per ten thousand can establish metastasis. Unfortunately, healthy cells in some organs express proteins on their surface that promote the seeding of cancer cells. Only in recent years have we learned how cancer can hijack neurons and neural circuits, while the poor nervous system is trying to police cancer's initiation and progression. Back in 2019, direct synapses were noted between a type of brain tumor and neurons, summarized in four words: "Gliomas are electrically active." In many ways, this finding, also seen in a companion paper on metastatic brain cancer, gave a solid foundation to the relatively new field of cancer neuroscience. Subsequently, a variety of neurotransmitters and nerve growth factors appeared to be involved in forming

membrane tube connections, not just in the brain but also in the spinal cord and peripheral nervous system. Certain driver gene mutations, like p53, can even reprogram nerve cells to make them secrete epinephrine and norepinephrine that can, in turn, stimulate tumor growth. The neuron–cancer cell connections can suppress the immune response. Reprogramming sympathetic nerves has been shown to promote T cell exhaustion and interfere with immune therapy for cancer. These new insights on the role of cancer cell–neuron connections have led to clinical trials across an array of cancer types, ranging from brain to breast, pancreas to prostate, with various repurposed drugs like propranolol, clonidine, or botulinum toxin.

The review of neuroscience and cancer by Rebecca Mancusi and Michelle Monje summarized the opportunity nicely: "Although targeting nervous system–cancer interactions may not by itself be sufficient to eradicate a tumor, this may be a necessary component of effective therapeutic regimens for currently intractable cancers such as high-grade gliomas and pancreatic cancers."

The cancer cell hijacking isn't limited to neurons. For decades, the ability of cancer to hijack blood vessels to create its own blood supply led researchers to develop drugs that block new blood vessel formation, known as antiangiogenesis agents, such as antibodies (bevacizumab, Avastin) and vascular endothelial growth factor, which have been approved and used for certain types of cancer, but with limited success. While we've long known of cancer's ability to evade our immune response, observing single cells revealed yet another dimension of cancer's hijacking behavior: it stole the mitochondria, powerhouse of the cell, from T cells! This is a double whammy, empowering the cancer cells while simultaneously dismantling the immune response, predominantly orchestrated via T cells. Like in the movie *Catch Me If You Can*, cancer cells can even switch their driver genes and cell identity during therapy. This has been seen in both lung and prostate adenocarcinoma.

The interactions between cancer and our gut microbiome play a pivotal role in our immune response. For example, when some patients with advanced, refractory solid cancers had a fecal microbiome transplant, it improved on the immunotherapy they were receiving. The totality of evidence to date strongly reinforces how critical our immune response is for preventing cancer's ability to

spread and colonize. Since our immune cells are less intact as we age (the jargon is immunosenescence), our health span is vulnerable. That's why it is essential to keep our immune system at maximum strength—and when the earliest detection of cancer threat is manifest, no less when it is clinically diagnosed, we need to ramp it up.

## THE HIGH-RISK PEOPLE NOW

The good news is that deaths from cancer have decreased, especially for breast cancer. In the United Kingdom, a study of more than five hundred thousand women with early invasive breast cancer showed a marked reduction in deaths from 1993 to 2015. Likewise, in the United States, the age-adjusted breast cancer mortality rate in 1979 was forty-eight per one hundred thousand women, which, in 2019, decreased to twenty-seven per one hundred thousand women, a 58 percent reduction. Much of this is ascribed to better treatment, since women who did not undergo mammography had improved survival, and there was no change in the contribution of screening over this extended forty-year period.

But the bad news is bad. New cases of cancer in the United States are on the rise, with over two million in 2023, and the trend is projected to continue. The rise is especially notable in young people, also documented in Europe and the UK, which alarmingly defies all we know about the predisposition of cancer with aging. Colon cancer is prominent now among young people (fig. 6.2). It is more likely to present in the late stages of the disease and involve the lower tract. It is the leading cause of cancer deaths in men under age fifty, and is the second-leading cause for women. People even in their teens and twenties are now presenting with advanced colon cancer.

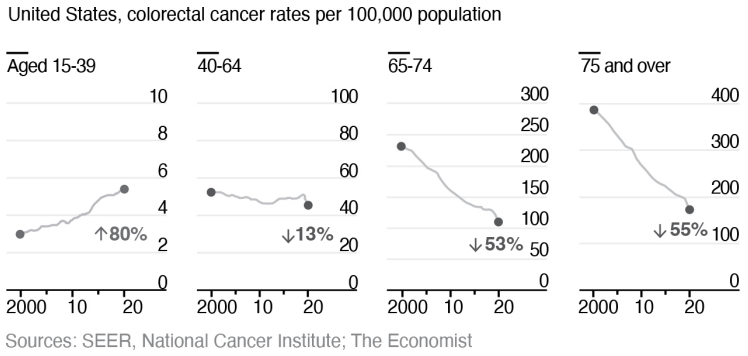



***Figure 6.2.*** Increase in colon cancer in younger age groups, United States. Adapted from "Bowel cancer is rising among young people," *Economist*, September 28, 2023, https://www.economist.com/graphic-detail/2023/09/28/bowel-cancer-is-rising-among-young-people. Description 30

The increase in cancer among the young is also noted for breast, uterine, and genitourinary cancers, and among ages thirty to thirty-nine years. In the United States, seventeen of thirty-four types of cancer are on the rise in younger birth cohorts, of which nine had previously been on the decline. I wouldn't be surprised if the root cause for some of this was, as suggested in the chapter on lifestyle+, high consumption of unprocessed foods, environmental carcinogenic exposures, or overuse of antibiotics. The latter may result from unintended alterations of the gut microbiome, with specific bacterial mutations that can help drive carcinogenesis. Half of the younger adults getting diagnosed with colon cancer are overweight; 17 percent are obese. Whether overweight or obesity contributes, or the other potential explanations mentioned are driving cancers in the young, remains to be resolved.

Globally, it has been projected that the number of new cancer cases will increase from twenty million in 2022 to thirty-five million in 2050, a huge 77 percent increase. The anticipated case breakdown for the United States in 2024 is seen in figure 6.3.

Listing cancer types by organ—which has been the only classification used for over one hundred years—exemplifies our unwillingness to acknowledge progress in the science. Cancers can now much more usefully be defined by their molecular characteristics, often agnostic to the organ in which they first showed up. Having sequenced the genomes of tens of thousands of tumors to identify driver mutations, and tagged these to treatment response, we have new ways to categorize cancer that are not yet part of routine medical practice. This is causing

unnecessary delays for treatment in millions of people. Patients with breast or gynecologic cancer had to wait seven to ten years before they could receive nivolumab (Opdivo), an immunotherapy monoclonal antibody that targets the PD-L1 protein, expressed at high levels by many cancers across organ types. Similarly, PARP inhibitors, such as olaparib (Lynparza) were shown to kill cells with BRCA1 or BRCA2 mutations in 2005. In 2014, olaparib was approved for ovarian cancer, but it took up to six years before it got approval for breast, pancreatic, and prostate cancers. With that delay, about two hundred thousand people who might have benefited lost their lives. We now have so many mutation-specific treatments, as I'll review, but the potential to apply these for people with cancer is too often lost, because they don't match up by the habitual organ classification. We frequently miss out because patients do not have full molecular characterization of their tumor. This is tragic at a time when sequencing costs have been dramatically reduced and when AI can often determine driver mutations alongside response to immunotherapy.

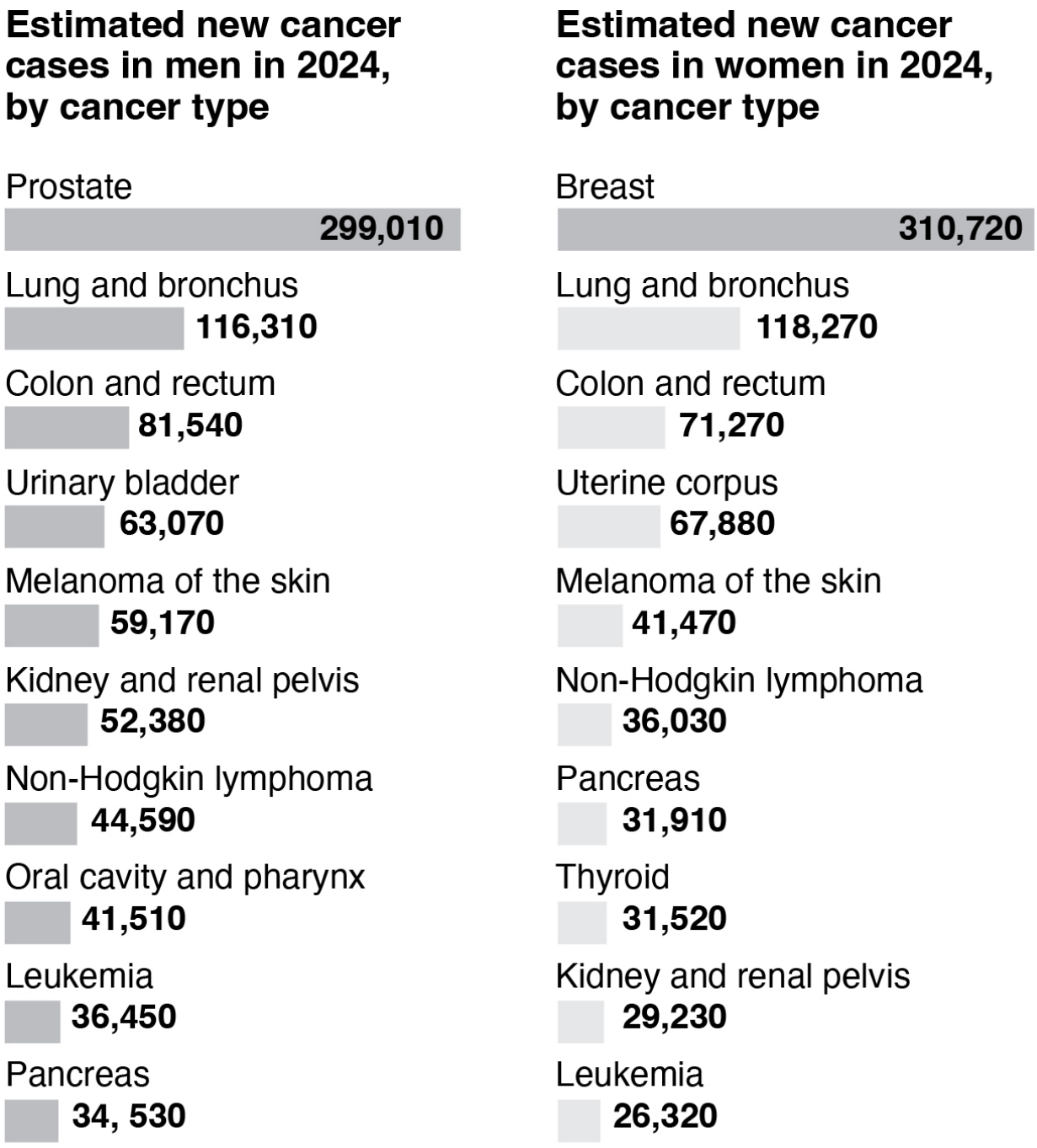



***Figure 6.3.*** New cancers by type and sex in the United States, 2024 cancer types. Adapted from Brianna Abbott, "Many cancers are on the rise in the U.S., even as overall deaths fall," *Wall Street Journal*, January 17, 2024, https://www.wsj.com/health/healthcare/cancer-deaths-rates-prevention-f73c82a4. Description 31

The one-size-fits-all approach is based on age, and it isn't working well. For five organ cancers—breast, colon, lung, cervical, and prostate—age is the singular criterion used for who should have screening. But only 14 percent of cancers diagnosed each year in the United States for the first four organ types are detected via screening. This comes at a cost of over $40 billion per year, a high rate of false positives, and marked controversy as to what should be the appropriate age for initiation, frequency, and cutoff. The data show how low a yield we see from our current approach of mass screening: only one person out of one thousand screened over ten years avoids death from that particular cancer.

Mammography for breast cancer screening has been shown to result in false positives in about half of women over the course of a decade even though 88 percent of women will never develop breast cancer. There is also the serious problem of overdiagnosis, unnecessarily detecting and often treating nonthreatening breast cancer, occurring in about one in three women aged seventy to seventy-four years, and nearly half of women aged seventy-five to eighty-four years. Overall, one in seven women aged fifty to seventy-four years has a breast cancer overdiagnosis. A meta-analysis of randomized trials screening tests assessing mammography, colonoscopy, prostate-specific antigen, and lung CT scans, involving over 2.1 million individuals followed for a median of ten years, did not show any improvement in lifespan. Adding to all these challenges is the sharp increases in young people who are developing cancer, often presenting at late stages, who are underage for the cancer screening guidelines.

The premise of screening to detect early cancer, well before it has had a chance to spread, is good. What we have now, with about half of cancers presenting at advanced stages 3 or 4, with metastasis, will not enable a population-wide improvement in health span. Fortunately, we have ample ways to reboot how we screen people who show no symptoms.

The first step is to determine whether they are at high risk (fig. 6.4).

That is the foundation for close surveillance of an individual and promotes the earliest possible diagnosis, when cancer begins to manifest, well before it shows up on a scan. This can now be achieved at an increasingly precise level, using abundant data sources and multimodal AI to integrate the many layers of data (fig. 6.4) that are beyond our conventional clinical risk assessment—beyond

merely considering a few factors like smoking or family history and coexisting conditions.

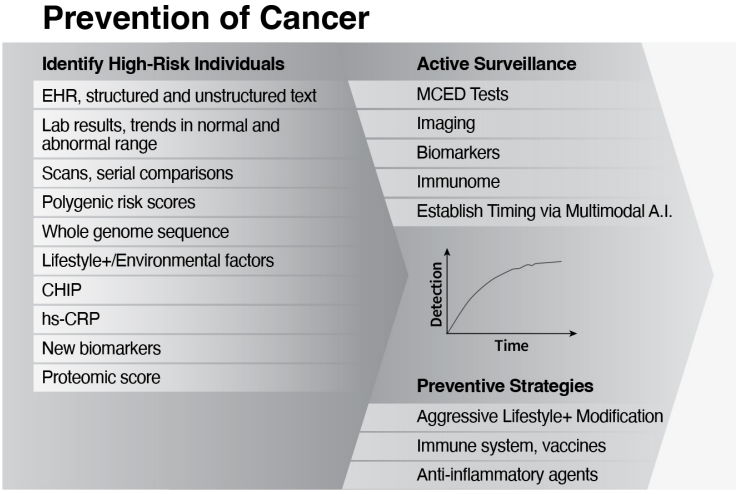



***Figure 6.4.*** Prevention of cancer. A multimodal AI approach of identifying high-risk individuals through comprehensive assessment, with active surveillance of these people, Multicancer Early Detection (MCED), and aggressive preventive strategies. Description 32

Risk partitioning capitalizes on AI's ability to "see" things that human experts can't. That includes systematically inputting the content from a person's electronic health records, which now, with generative AI, includes the unstructured text that often provides essential content. A person's serial lab tests and medical scans may tell an important story when they were previously deemed normal but, when assessed longitudinally, indicate a trend in the wrong direction. Moreover, there are now hundreds of studies that have validated the power of AI to augment the accurate interpretation of scans. We can better avoid missing a lung nodule on a chest X-ray. Machine eyes can pick up abnormalities that expert radiologists wouldn't even look for, like in the pancreas on a chest CT scan. The genetic data, either in the form of polygenic risk scores or a whole genome sequence (from which these risk scores can be derived), provides key, independent information, as do emerging biomarker blood tests.

As you may recall, a polygenic risk score can be inexpensively obtained from a gene chip (array) that assesses over a million common genomic variants that have been quantitatively validated to be associated with risk of a particular cancer, in terms of a score. While these were initially based on European ancestry cohorts,

their accuracy and clinical utility have been markedly improved with diverse ancestry and large population data. That's why some health systems have begun clinical implementation, despite some skepticism among researchers who continue to call for randomized trials to determine whether people having these scores saves lives. My view is that such trials are not likely to get done, and even if there was a funding commitment, it would take many years of follow-up. The principal goal of a polygenic risk score is to identify high-risk individuals so that they can be followed closely. Even if some people wind up getting surveillance measures that prove to be unnecessary (a false positive), that's better than the one-size-fits-all current approach. Polygenic risk scores can miss high-risk cases, which is why it is so important to have multiple layers of orthogonal (complementary, additive) data, as shown in figure 6.4. The Mass General Brigham health system in Boston has begun providing polygenic risk scores for its patients. In published results from more than thirty-six thousand participants of diverse ancestry, 8.6 percent were found to be at high risk for breast cancer, 5.4 percent at high risk for colon cancer, and 13 percent at increased risk for prostate cancer. All were at more than double the risk of the general population, with some variation by ancestry. Polygenic risk score data from Finland's FinnGen databank of over 450,000 individuals has indicated when colon cancer screening should be initiated, with the highest risk (top 1%) at age forty-nine years and the lowest risk (20%) at age sixty-seven years. That would save most people several colonoscopies! Similarly, partitioning by low polygenic risk score among more than twenty-five thousand women in the Healthy Nevada project indicated the potential to defer mammography by five to ten years, for those with a more than 60 percent reduction of risk.

The case for routine whole genome sequencing to determine risk of cancer keeps getting stronger. Whole genome sequencing of our three billion letters can now be done for about $200. We've known about well over a hundred cancer predisposition genes for over a decade that would all be detected by sequencing. In Iceland, nearly 58,000 people underwent whole genome sequencing, more than 108,000 people had a gene chip, and 4 percent were found to have actionable disease-causing gene mutations, nearly half of which were accounted for by cancer gene mutations (such as BRCA1, BRCA2, PALB2, MSH6, PMS2)

for high risk of breast, ovarian, prostate, pancreatic, or colon cancer. Similar frequencies of these cancer gene mutations were found in the UK Biobank and other cohorts. The Icelandic data is unique because it has data from death certificates, and it shows carriers of cancer-causing variants died three years sooner. In the Icelandic population, there is a founder mutation in BRCA2, which was linked to a seven-year lifespan reduction, and the overall analysis was performed without including this founder mutation. As the accompanying editorial pointed out,

> By the age of 65 years, 10 percent of the participants with pathogenic or likely pathogenic variants in cancer genes had died. In contrast, in the group of participants without such a variant, 10 percent had died by 73 years of age.

Of note, results were analyzed from only seventy-three genes, which are currently considered by the American College of Medical Genetics and Genomics as actionable but represent a tiny (0.3%) proportion of the approximately twenty thousand genes in our genome. The Healthy Oregon project of whole genome population sequencing included some additional cancer genes in its reporting and found 5 percent of its cohort had a pathogenic or likely pathogenic cancer gene mutation. An assessment of population sequencing for three common hereditary conditions—breast cancer and ovarian cancer, Lynch syndrome, and familial hypercholesteremia—was found to be cost-effective for people younger than forty.

Some biomarkers can be used that are associated with a higher risk of specific cancers, such as proteins for risk of pancreatic cancer or a circulating microbiome DNA for lung cancer. Clonal hematopoiesis of indeterminate potential, clones of blood stem cells with driver gene mutation frequency ≥2 percent, is one such example, and it can be derived through genome sequencing. Its presence, which is increasingly common with aging, carries an elevenfold risk for blood cancer and increased risk of lung cancer and nonmelanoma skin cancer. Like so many other potential biomarkers, CHIP is featured in hundreds of research publications in leading biomedical journals but has yet to find its way into the clinic as a marker of increased risk. Similarly, inflammatory markers like high-sensitivity C-reactive protein (hs-CRP) are not used to assess risk of cancer, despite supportive data,

and a large trial of a monoclonal antibody directed against a key protein interleukin-1β, to suppress inflammation, significantly reduced fatal cancers and lung cancer, even though it was designed to reduce cardiovascular events. A main criterion for enrollment of the ten thousand participants was an elevated hs-CRP. The results tell us that directly measuring inflammation markers in the blood for directing anti-inflammatory treatment has considerable potential to lessen the toll of cancer. It also represents something else we don't do today and are not pursuing with better biomarkers and drugs.

If we could assess a person's immune status with a comprehensive test—what I call an "immunome"—assaying our innate and adaptive immunity in depth, it would help us detect individuals with impaired responsiveness, as typically seen with aging. That could trigger use of a vaccine or other strategy to strengthen the immune system in a person at high risk. The only immune marker we have in common practice today is the ratio of white blood cells to lymphocytes, which is rudimentary but predictive of outcomes across all major diseases and in patients with cancer.

Pancreatic cancer is renowned for being diagnosed far too late, with a high fatality rate. The five-year survival rate is less than 10 percent. This hit home recently when my seventy-three-year-old brother-in-law in good health presented to an emergency department for evaluation of acute blurred vision. He was admitted to the hospital and an extensive workup determined that it was due to blood clots from stage 4 pancreatic cancer, for which he had no symptoms or warning. Approximately fifty thousand deaths in the United States are from pancreatic cancer each year, making it the third-leading cause of death from cancer after lung and colon.

In light of that, a major study was conducted in two distinct cohorts: more than eight million people in Denmark and about three million people in the US Veterans Affairs health system. A total of nearly twenty-eight thousand cases of pancreatic cancer were diagnosed. When an AI model combed through all the electronic medical records over several years, it achieved high accuracy for predicting who would get pancreatic cancer. For the highest-risk group, 320 of 1,000 people aged fifty and older developed pancreatic cancer. In a similar study of fifty-five health care organizations in the United States, AI analytics of the

electronic records differentiated the 35,000 patients who developed pancreatic cancer from 1.5 million people who did not. In both studies, the model identified tens of features, such as results of lab tests, symptoms, medications, and coexisting conditions, that collectively accounted for its performance. That's just with the use of time-sequenced electronic records.

What if we add other layers, such as genome sequencing, scans, or blood biomarkers?

If whole genome sequencing was routine, we wouldn't miss data from high-risk pancreatic cancer predisposition genes such as mutations in BRCA1, BRCA2, PALB2, CDKN2A, ATM, Lynch syndrome (MLH1, MSH2, MSH6, PMS2, and EPCAM), Peutz-Jeghers syndrome (STK11), and Li-Fraumeni syndrome (TP53). Using genomics of the tumor and AI analytics, it is possible to determine a more successful treatment plan and predict recurrence of a tumor more than ten years from initial diagnosis.

Many patients get CT scans of the chest or abdomen without contrast dye for various reasons that have nothing to do with assessment of the pancreas. But an AI model was trained on both types of CT scans to accurately detect pancreatic cancer from these scans, markedly outperforming radiologists, a task considered so difficult that it would defy attempts from a noncontrast scan. This work was independently replicated with ability to pick up preinvasive pancreatic cancer from standard abdominal CTs with high accuracy. The compounds carbohydrate antigen 19.9 (CA19-9) and bilirubin have been shown to correlate with pancreatic cancer and differentiate from benign pancreatic abnormalities. We'll come back to pancreatic cancer shortly for other layers of data to predict optimal treatment and patient outcomes.

Now let's consider how AI is making a difference for breast cancer. Recall that only 12 percent of women in the United States will ever manifest breast cancer in their lifetime. If we could determine who is at risk, it could save the vast majority frequent screening, false positives and the follow-up scans and biopsies they engender, no less the angst and cost that arises from them, and overdiagnosis.

One way that has been pursued is partitioning based on the mammogram, for which AI can provide important prognostic information for five-year risk. That brings up another key point. Just knowing a person is at risk during a lifetime

isn't enough—we also want to know more about the likelihood of when a cancer will first manifest. A mammographic texture model assessed in over 280,000 screenings in the Netherlands and Denmark found the highest 10 percent risk of women developed about 40 percent of the breast cancer cases during follow-up. A mammography AI model assessed in a four-country case-control study in Europe identified women at high risk who were told their mammogram was normal. In a study conducted at four centers in three countries, the United States (Mass General and Emory), Sweden (Karolinksa), and Taiwan (Chang Gung), a mammography AI-based model for detecting future risk was far more efficient—as defined by reduction in number of mammograms and earlier detection—than routine, age-based screening.

But just pinning risk stratification on the mammogram or, if performed, ultrasound or MRI, is only one dimension. Other layers of data would include lifestyle+ (obesity, alcohol consumption, exercise), polygenic risk score, genome sequencing, reproductive factors such as age at menarche, age at menopause, gravidity, parity, and age at first birth, and breastfeeding. Such a multimodal model has not been prospectively assessed, but it would be expected to help partition low, intermediate, and high risk and supersede age-based mass screening in the future. Many prospective clinical trials of tailored screening are in progress.

## TESTS OF THE TINIEST

While the multiple layers of data can certainly help to pick up people at high risk for cancer and develop smarter screening strategies, tests that directly measure microscopic evidence of cancer in an individual have drawn intense interest. The imprecise term *liquid biopsy* has frequently been used but isn't quite right. Various tests seek evidence of cancer such as cell-free tumor DNA in the blood plasma, fragmentation of tumor DNA, methylation of DNA, circulating tumor cells in the blood, circulating exosomes, proteins, metabolites, cell-free tumor DNA in the urine or stool, cell-free RNA tests, repeat DNA elements, and alterations of T cell receptors. Such Multicancer Early Detection (MCED) assays are constantly getting refined. For example, the amount of cell-free tumor DNA in a blood sample is very limited, but priming agents can be added to reduce its

clearance and improve accuracy of testing. Deep-learning AI has been shown to overcome the low-quantity problem with a radical signal-to-noise enrichment, making these assays ultrasensitive. One of the most difficult cancers to diagnose early is ovarian, but AI of DNA methylation markers has been shown to improve on our current assessment.

There are also assays that detect certain proteins or metabolic markers, such as glycosaminoglycans, that indicate a higher likelihood that cancer may be present, as well as blood tests that combine cell-free tumor DNA and proteins in a panel.

The test with the most extensive clinical experience to date is known as the GRAIL Galleri. This blood test interrogates DNA methylation via bisulphite sequencing for about one hundred thousand sites that are associated with cancer. It uses AI to determine whether there is a "cancer signal" and, if present, to help localize its likely organ source. In a prospective clinical trial of over sixty-six hundred participants aged fifty years and older, 92 percent of whom were white, a cancer signal was found in ninety-two individuals (1.4%). Of these, thirty-five individuals (0.5%) were found by additional workup to have cancer, which is a positive predictive value of only 38 percent. The AI prediction of cancer site of origin accuracy was 97 percent. Given the diverse sites of origin, including bone, head and neck, ovary, liver, uterus, blood, breast, lung, and gastrointestinal tract, that's quite good. However, the small number of cancer cases detected were mostly at stage 3 or 4, already metastatic.

The fifty-seven individuals with a false-positive cancer signal indicate a low false-positive rate (specificity of 98%). Of interest, it is possible that the abnormal cell-free methylation signal in some individuals may reflect a transient appearance of microscopic cancer that was quashed by the person's immune system. Anecdotes of individuals who had a false positive and on repeat testing were negative suggest longer-term follow-up to see how common this is. Strengthening our immune system might be a preventive strategy worth pursuing.

The problem with this test that is marketed for $949 for people aged fifty and older is that the yield is remarkably low. Only five per one thousand people test positive, and, of these, just two per one thousand were actually diagnosed early, in stage 1 or 2, before the cancer spread. The proportion of false negatives among every one thousand people tested is unknowable, but, and I'm sorry to say it,

anyone who gets back a report of "no cancer signal detected" should not have confidence that they are not incubating a cancer.

The detection rate for early cancer (stage 1 and 2) is poor compared with later stages. Picking up cancer at the microscopic stage, before it can be seen on a scan or induce symptoms, or potentiate spread, is the key to improving outcomes, using interventions to change the natural history of cancer progression. Simply put, detection is not equivalent to favorably altering outcomes. That is why randomized trials are necessary, such as one by the National Health Service in the UK of 140,000 participants and the National Cancer Institute in the United States of 225,000 participants. These very large trials of healthy, asymptomatic people are again using age as the main inclusion criterion. It will be years before such trials will be complete for clinical outcomes, and there will be questions about the diversity of the participants, since we know the occurrence and behavior of many cancers are shaped by a person's ancestry. But the problem of lacking compelling evidence from randomized trials is perhaps not the biggest problem. None of the tests are yet FDA approved or reimbursed by insurance carriers.

Unfortunately, to date these tests are following the mass screening template of using age alone rather than integrating all an individual's data to determine risk. There's also the potential of combining tests to improve accuracy, such as cell-free tumor DNA sequencing and methylation, but that will raise the cost. One company that uses the fragmentation DNA assay has incorporated polygenic risk scores with its assessment, without added cost, which represents a step in the right direction.

For now, these tests are for the curious affluent and only carry potential value if positive, and only about half of those will be indicative of an actual cancer diagnosis that was not otherwise apparent. It will be primarily used by the same people who get executive health physicals with all sorts of tests that have not been validated in healthy people, at exorbitant costs.

That's similar—without adequate proof of benefit—to the current use of whole-body MRI tests that are being heavily marketed. In the United States, Prenuvo and Ezra offer this for $2,500, with multiple endorsements by celebrities, like Kim Kardashian, and long waiting lists. Instead of theoretically spotting

cancer at its earliest microscopic stage, MRI can only detect cancer once it exceeds a certain mass size; it's macroscopic and even less likely to find a tumor early than the micro testing we've been examining. As with the person with a cancer signal from the Galleri test, an abnormal MRI sets off a cascade of more tests to determine whether there is cancer present, which can culminate in an organ biopsy, carrying risk. The chance that an abnormal MRI could promote "incidentalomas," benign findings such as a nonmalignant nodule or cyst, is high: a meta-analysis of all the studies indicates that it occurs in at least 19 percent of people. That, along with the lack of sufficient evidence, is why the American College of Radiology recommends against total body–screening MRIs.

Dhruv Khullar, a young physician, underwent a whole-body MRI and was found to have a prostate lesion, which led to blood tests and a dedicated prostate MRI, and close surveillance that will include serial imaging and perhaps a prostate biopsy (and serial biopsies) in the future. Khullar's physician told him, "Prenuvo probably views your story as a success—I view your story as a tragedy. They've created in your mind this uncertainty. You were a healthy person, and now you've become a patient."

Both micro and macro tests such as whole-body MRI can help people; the tests have detected cancer in some healthy people that led to definitive treatment. However, is there a net benefit or harm in your case and generally in the population? We don't yet know, and we won't until randomized trials assess clinical outcomes. At least for the micro tests, such randomized trials have been initiated, which will fill gaps in the evidence. Less helpful, Prenuvo launched an observational study of one hundred thousand people undergoing whole-body MRI over the next ten years.

While those gaps are being worked out, we do know that exploiting deep-learning AI interpretation of many medical scans and pathology slides delivers more accurate diagnoses. For medical images, the two most studied are mammography and colonoscopy. The largest randomized trial in medical AI to date was performed in Sweden, assessing radiologists' interpretation of mammograms in eighty thousand women participants, with or without AI. The use of AI increased screen-detected cancers by 15 percent and reduced the workload by 44 percent. In Denmark, among about sixty thousand women

screened with mammography before and after AI was implemented, there was improved cancer detection and fewer false positives, and the radiologist's workload was reduced by a third. Other large prospective studies have shown similar results. This has also been replicated in the real world, in Hungary, with 13 percent more breast cancer diagnoses being made with the combination of AI and radiologists. In the United States, more than two dozen AI algorithms have been FDA cleared or approved for breast cancer detection, one of which is RadNet's algorithm that compared the radiologist's interpretation to the radiologist plus their AI (which includes a 3D reconstruction) and showed a 14 percent increased detection rate. However, none of these algorithms have billing codes or are reimbursable by insurance providers. When this was offered at RadNet's 350 imaging centers distributed throughout the United States, women were asked to pay an additional $40 out of pocket for their AI mammogram interpretation. There's another big issue: we don't yet know whether this boost in detection will change outcomes for the people getting diagnosed. The big Swedish multiyear follow-up randomized trial will clarify what difference such detection makes for breast cancer prevention.

Over thirty-three randomized trials of colonoscopy compared gastroenterologists working alone or accompanied by real-time machine vision to pick up tumors of the colon or rectum, known as adenomatous polyps. The clear result: use of AI reduced missing polyps by more than 50 percent. In parallel with mammography, diagnosis of polyps is not the same as altering the natural history of colon cancer. The implications for breast and colon cancer imaging extend to many other organs, such as picking up lung nodules on chest X-ray or CT scans that may be cancerous, interpreting a prostate MRI or ultrasound for prostate cancer, diagnosing likely kidney cancer from an abdominal CT, and detecting pancreatic cancer from an abdominal or chest CT scan. For these cancer-imaging studies, the results are very encouraging for improved accuracy of cancer detection.

AI has many biomedical applications. Pathology samples are typically stained by hematoxylin and eosin on glass slides and assessed subjectively by pathologists for possible presence of cancer, benign or malignant, and tumor type (organ of origin). That unfortunately leads to some important inconsistency between

readers and is rather limited in scope for what now can be achieved with an analysis of digitized whole slide images.

The progress that has been propelled by AI for interpretation of histopathology slides is striking. AI deep neural networks can accurately identify driver genomic mutations, locate the site of origin, articulate the prognosis, predict drug response, and provide treatment recommendations to an extent not previously possible. The accuracy and applications across the full range of cancer types, by both organ and molecular features, has markedly expanded with multimodal AI and the new generative AI (large language models), which now can incorporate data from single-cell sequencing, spatial omics, the tumor microenvironment, imaging, and the immune response. Such a rich depth of information indiscernible to expert human eyes, but "seen" by machine eyes, has transformed how pathology specimens should be assessed. As pathologists Ali Bashashati and S. Larry Goldenberg at the University of British Columbia recently asserted, "AI will not replace pathologists—it will only replace those who do not use AI in their daily practice."

## HEALTH SPAN TREATMENTS

Now that we've seen how screening can be upended, how accuracy of diagnosis can be improved, we can see the same principles apply to an overhaul of treatments. For many decades, we've heavily relied on toxic chemotherapy that nonspecifically kills our cells that divide frequently, making us sick with gastrointestinal distress, bald, and suffering with chemo-brain. Such treatments are so mutagenic they may even induce a new cancer. We're on the road to far more intelligent, focused approaches to quash cancer and spare our healthy cells and tissue.

To reiterate, the molecular characterization of a person's cancer is vital, with so many mutations providing guidance for approved treatments. Remember, there are genes and then there are the proteins they make. Some of these proteins are growth factors that play a critical role in the development of cancer. Genes with important mutations that drive predisposition to cancer include HER2, BRCA1, BRCA2, EGFR, NTRK, ALK, BRAF, ERBB2, FGF, KIT, MET,

RET, ROS, PIK3CA, PDGF, PD-L1, and more. Even KRAS, a frequent driver mutation of gastrointestinal and other cancers, once considered undruggable, has FDA-approved treatments that appear to be quite effective. More are on the way, including, perhaps, molecular glues. In patients with advanced non–small cell lung cancer who carry the ALK mutation, five-year survival was improved from 8 percent with traditional therapy to 60 percent using lorlatinib, a pill targeting the ALK and ROS1 genes. Beyond specific mutations, examining the genome sequence for important markers such as microsatellite instability, mismatch repair deficiency, homologous recombination deficiency, and tumor mutational burden provides insight about the use of specific, bespoke FDA-approved drugs. Some of the mutation-targeted drugs have been proven to shrink or eliminate tumors (as observed by scan) but, it must be said, without substantially improving cancer survival.

In contrast, mutation-directed treatment was successful in advanced breast cancer in women who had "HER2-low" tumors. A person may have too little of the HER2 growth factor and develop HER2-low cancer (about 55% of breast cancer patients), or a person may have too much of this particular growth factor and develop HER2-positive cancers (about 17% of breast cancer patients). HER2-positive breast cancer already had effective treatments, but HER2-low has been a challenging puzzle. A randomized trial of the drugs trastuzumab deruxtecan versus untargeted therapy (T-Dxd) in more than 550 HER2-low patients, who had failed previous therapy, led to significant improvement of survival, and progression-free survival.

This is noteworthy since there was an average seven-month improved survival, which contrasts to the expectation of only weeks or no benefit in survival for such patients. T-Dxd is the prototypic antibody-drug conjugate, essentially the drug Herceptin linked to a chemotherapy drug so the latter can achieve high concentration in the tumor, less release throughout the body. That enhances efficacy while also reducing side effects. How could this marvelous success have been achieved?

The drug is called a conjugated (two molecules joined together) antibody because it uses an immunotherapy (trastuzumab) to attach to the cancer cell and deliver the deruxtecan, chemotherapy drug, to get into the cell to destroy it.

These “weaponized antibodies” are having unprecedented success for challenging tumor types, such as using vedotin for bladder cancer, or tebentafusptebn for malignant melanoma, EGFR-HER3 for several advanced solid tumors. Over twenty such “biological missiles” have been approved by the FDA, and many more are in ongoing clinical trials.

Targeting tumor protein P53 (TP53), the most common cancer driver mutation, but without an effective treatment, may even be possible using a conjugate strategy. An antibody-drug conjugate targeting a blood stem cell marker is an exciting new approach to treat blood cancers and autoimmune diseases. This class of drugs is associated with some unique toxicities of the lung, eye, and skin that must be carefully monitored.

The antibody-drug conjugates build on our current immunotherapies that power up our immune system to fight cancer by releasing the brakes of T cell activation, a process that can be suppressed by cancer cells. T cell activation is a multipronged body defense process whereby T cells spot intruders (such as a cancer cell). A molecular signal calls in the troops for more T cells, which multiply by the millions and differentiate into other types of immune cells to eliminate the intruder. T cells and their helper cells then release proteins called cytokines, which can kill cancer cells.

The mainstay of our cancer-directed immunotherapies are checkpoint inhibitors, blocking the part of our immune system that constrains the T cell response. A more robust, unbridled activation of T cells results. The list of checkpoint inhibitors includes pembrolizumab (Keytruda), nivolumab (Opdivo), and cemiplimab (Libtayo) that target programmed cell death protein 1 (PD-1); atezolizumab (Tecentriq), durvalumab (Imfinzi), and avelumab (Bavencio) that target programmed cell death ligand 1 (PD-L1); Keytruda, Opdivo, and Loqtorzi that block the interaction between PD-1 and PL-D1; Ipilimumab (Yervoy) that blocks CTLA-4; and Opdualag (a LAG-3 checkpoint inhibitor, retalimab-rmbw, combined with nivolumab). One trial of PD-1 blockade for advanced colorectal cancer is notable. All among a small group of patients with mutations in genes that correct errors in DNA replication, known as mismatch repair-deficient, had a pronounced clinical response with no tumor detected by follow-up imaging. However, this result is an exception—most checkpoint inhibitor interventions

only yield short-term success. There is also a group of antibodies called bispecific T cell engagers (BiTEs), which have a linker, like the antibody-conjugate drugs, to bridge tumor and T cell recognition, power up the immune response, and kill cancer cells. Several BiTEs are now approved for blood cancers, and more are in clinical trials.

Checkpoint inhibitors and antibody-conjugate drugs are only a couple of the enhancements that are ramping up our immune response against cancer. Modified oncolytic viruses can infect the tumor, changing it immunologically from "cold" to "hot," activating inflammatory responses, with or without a checkpoint inhibitor. While they had a checkered track record for success, recent reports in bladder cancer and other refractory tumors are promising.

Not only are there antibody-drug conjugates but also cell-drug conjugates. Chemotherapy or immunotherapy drugs, such as interleukin-15 or interleukin-2, can be attached to T cells, red cells, and platelets on clinical trials of various cancers.

It has become clear that our current cancer immunotherapy approaches are helping but are all too often insufficient to achieve durable success. The addition of Janus kinase inhibitors (JAKs) combined with checkpoint inhibitors led to increased success against refractory Hodgkin lymphoma and non–small cell lung cancer. The mechanism of benefit, "JAK-ing" up the immune response, appears to be blocking the immunosuppressive effect of interferons secreted by T cells, restoring their ability to kill cancer cells.

We know the gut microbiome constituents have a substantial influence on the success of cancer immunotherapy, but it is not assessed in patients with cancer, no less ways to modulate it with prebiotics, probiotics, or bacterial consortia (introduction of many strains). Clinical trials are underway to test such interventions.

Two platforms that further leverage our immune system—vaccines and engineered cells—are concurrently emerging, substantially building on the early success of immunotherapy. Cancer vaccines in the form of DNA, mRNA, or peptides, packaged in nanoparticles, cells, or naked, are being used to induce a higher-grade immune response. Using repeat shots, the goal is to train a person's immune system to recognize tumor cells and destroy them. The vaccines are

made against proteins on the cancer cell surface known as neoantigens or target commonly mutated variants of driver genes such as KRAS or TP53. The "vaccine on demand" process for making these customized vaccines is interesting, as described by Elizabeth Sullivan, head of operations for the personalized vaccines program at Moderna:

> Each bay houses its own "single use personalized RNA+" machine, a refrigerator-sized unit that cranks out long strands of mRNA encoding up to thirty-four specific cancer mutations. The mutations correspond to different neoantigens, neatly arranged in a sequence. A mixing device then encapsulates the mRNA in fatty nanoparticles to enhance its stability and cellular uptake.

And of course, AI helps identify the ones that suit the particular individual.

A personalized vaccine known as mRNA-4157, coming in an mRNA nanoparticle package just like the COVID vaccines, has in a randomized trial proven more effective in combination with pembrolizumab than pembrolizumab alone in battling melanoma. A three-year follow-up of an mRNA nanoparticle vaccine for pancreatic cancer that was personalized using antigens found in a resected tumor showed a persistently elevated immune response and delayed recurrence. A similar approach has been used in patients with non–small cell lung cancer, directed toward stimulating dendritic cells, a type of immune cell. Rejuvenating the aged immune system's dendritic cells to eradicate tumors in old mice begs for testing such an approach in people with cancer. The extensive number of RNA-based vaccines in ongoing clinical trials for nearly all solid tumor types is notable.

Such results are telling us that there is plenty of room for improvement by maxing out the immune response to cancer via combinations of approaches. One that is attracting considerable attention is engineering T cells known as CAR-T for chimeric antigen receptor (CAR)-T cells. It's cell therapy for cancer that already has multiple FDA approvals for different blood cancers, with more than forty thousand patients treated in the United States alone. Solid tumors, which account for the vast majority, are not yet responding as well to this approach, but progress is being made.

CAR-T therapy involves removing the T cells from a patient with cancer, sequencing the cell's DNA and RNA, and using that information to specifically activate the T cells against the person's cancer, then reinfusing the cells back to

the patient. This, along with the use of personalized neoantigen vaccines, can be considered the quintessential individualized medical intervention. In the first patients receiving CAR-T cells delivered to the brain for recurrent glioblastoma, one of the most fatal cancers known, there was rapid and dramatic regression of tumors. That type of excellent brain tumor response was also seen with an intravenous administration of an mRNA with lipid particle aggregates. It is remarkable to see how engineered T cells and cancer vaccines have evolved together. They have broadened the ways we can improve a person's immune response to cancer. The number of combinations of different ways to fire up the immune system to fight against cancer is astounding, now including CAR-T, oncolytic viruses, antitumor antibodies, vaccines, BiTEs, activating dendritic cells, gut microbiome modification, and small molecules. It is hard to grasp just how much better we are becoming at deploying the most bespoke treatment for a given individual.

There are currently more than five hundred clinical trials of CAR-T therapy to approach solid cancer, trying to override the challenges of getting the engineered T cells into the tumor, much more diversity of mutations, and overcoming the local suppression of the immune response. But scientists are pursuing many ways to override these challenges, including CRISPR genome editing to activate thousands of genes in the T cells or express proteins with enhanced cell penetration. They are also aiming to rejuvenate T cells that are considered exhausted or dysfunctional because they've lost their ability to fight cancer cells. These are new strategies to enhance their fitness or ensure they are better protected (cutely called "armored" CAR-T cells). Researchers are exploring the use of naturally occurring mutations, not present in an individual's cancer, to add to T cells as a means of revving up the immune response. And we're learning about injecting a virus that specifically infects T cells to insert the genes needed to achieve T cell engineering within the body. On and off "suicide" switches can be programmed into the cells to limit cytokine release, a prime toxicity generator.

In addition to T cells, scientists are engineering other immune cells, such as CAR-macrophages and CAR-NK (natural killer cells). Current engineering of cells is laborious and expensive (estimated cost now at $500,000) since it involves

removing cells from the person's body, modulating them, and returning them. There is now considerable effort to move to off-the-shelf cell availability, by manufacturing cells that have powerful immune defense properties but are not derived from or specific to a patient. Such a strategy could markedly cut costs and time to treatment. In India, they are being provided at about one-tenth the cost. In Australia, Interius Biotherapeutics has initiated the first clinical trial of in vivo CAR-T, with an intravenous infusion of an inactivated lentovirus to insert antigens onto T cells and transform a person's own immune cells to fight cancer. With the intensity of efforts and multiplicity of strategies, it is likely that we'll see engineered cells become a mainstay of treatment for solid cancers in the years ahead.

There are a small number of T cell cancers that have cropped up after CAR-T therapy, about one per one thousand people treated, and via molecular markers, most appear to have been induced by the T cells, because the chimeric antigen receptor gene can be detected. Besides engineering T cells derived from a patient's blood, there are also tumor-infiltrating lymphocytes, isolated and grown from a patient's cancer tissue, the first of which was FDA approved in 2024 for advanced melanoma. Potential treatments for many other types of solid cancer are being pursued in clinical trials.

Which patients might benefit from which therapy, or what is emerging as multitudes of combinations of therapies, is being pursued with AI by integrating all of a patient's data, to improve clinical trials testing the interventions, with multiple start-up companies that have cropped up for guiding clinicians on patient selection. Knowledge of how influential the gut microbiome is in cancer, and how that can be modulated, is laying the foundation for an improved, individualized approach. Use of a patient's cancer cells in culture to form organoids can help predict response to therapy. Radiation therapy for cancer is one field that has been undergoing AI transformation, specifically decisions on treatment, planning, and delivery of radiotherapy.

There's far-reaching potential for the use of digital twins to determine optimal cancer treatment at the individual level in the future. That requires setting up a huge dataset with hundreds of thousands, if not millions, of patients who have been diagnosed and treated for cancer. It would include their electronic health

records, labs, medical scans, pathology whole slide images, genomic data, liquid (micro) biopsy results, treatments, and outcome. This multimodal data serves up the potential for a newly diagnosed patient, by nearest neighbor AI analysis, to have their digital twin(s) identified and so have confidently predicted what treatment will work because it has previously worked in the twin. Such a strategy would be complementary to randomized clinical trials. Of course, no twin is perfect. Every cancer has unique features at the molecular level, and every individual is unique.

But in comparison to the heterogeneity of participants in cancer trials and their variable responses to treatment, a digital twin is a great potential improvement. There is no such resource built to date, except for very small ones for a single type (by organ) of cancer. We'll only be able to know the effectiveness of digital twins when they are fully built at scale and we can track outcomes of patients whose treatments were informed by this strategy. Barriers for building such a data resource include concerns over privacy and security of the data, along with the funding requirements for algorithmic development, validation, and data acquisition with automated longitudinal participant data inputs. Eventually, we're likely to see the infrastructure for digital twins develop, but, unfortunately, the near-term prospects remain remote.

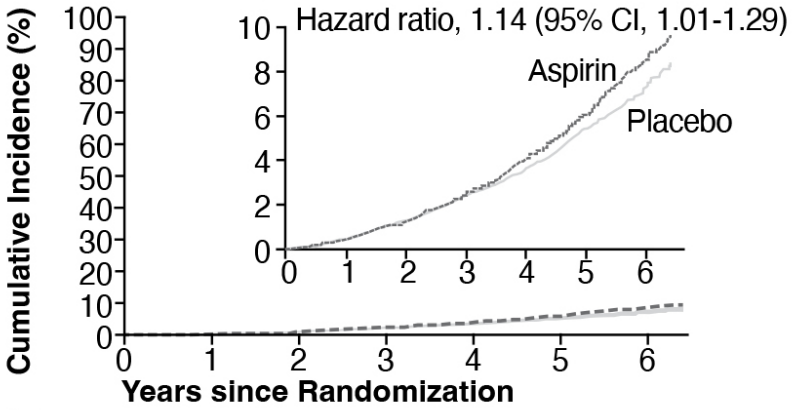
## PREVENTION

Testing current strategies to prevent cancer are yet another dimension of the need to reboot our approach. Take the example of low-dose aspirin to prevent cardiovascular disease and colon cancer, once widely recommended, and still taken by at least twenty-nine million Americans when last assessed. In over nineteen thousand participants in a randomized trial of low-dose aspirin (100 mg) or placebo with five-year follow-up, there was a significant excess of all-cause deaths (14% more), deaths from cancer (31% higher), and major bleeding events (38% higher) in the aspirin group (fig. 6.5).

Furthermore, there was no reduction in cardiovascular disease or disability-free survival. While that trial predominantly enrolled people aged seventy and older, another even larger randomized trial in men aged fifty-five and older, and

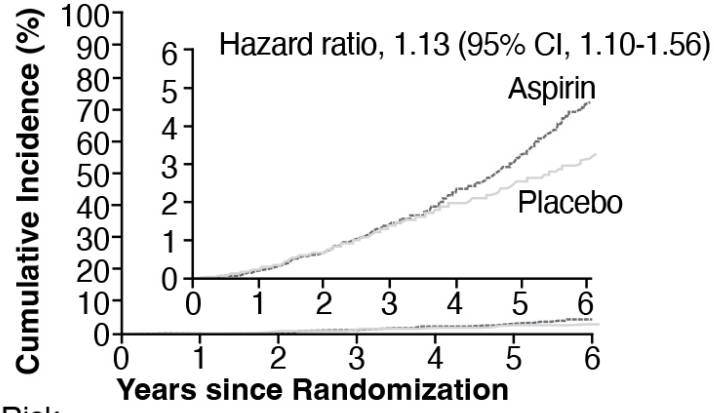
women sixty years and older, found the same lack of protection against cardiovascular disease and significant excess of hemorrhagic events. In 2022, four years after these and other trials with compelling evidence were published, the US Preventive Services Task Force stopped recommending aspirin to prevent colon cancer or cardiovascular events. In their guidelines, the hedge for cardiovascular events was that for people aged forty to fifty-nine years, "clinicians should decide for themselves." How fuzzy is that, and how much better could we do if we precisely defined risk before testing an intervention, and recommending it at mass scale, based on just one factor—age?

## All-Cause Mortality



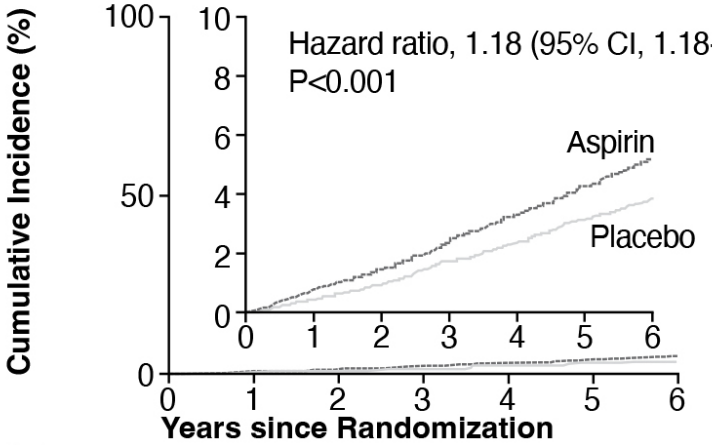

| No. at Risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aspirin | 9525 | 9481 | 9408 | 8286 | 6291 | 4016 | 1495 |
| Placebo | 9589 | 9545 | 9466 | 8369 | 6367 | 4077 | 1476 |

## Deaths from Cancer




| No. at Risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aspirin | 9525 | 9481 | 9408 | 8286 | 6291 | 4016 | 1495 |
| Placebo | 9589 | 9545 | 9466 | 8369 | 6367 | 4077 | 1476 |

## Major Bleeding Events




| No. at Risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aspirin | 9525 | 9337 | 9094 | 7833 | 5826 | 3574 | 1248 |
| Placebo | 9589 | 9424 | 9192 | 7930 | 5935 | 3632 | 1244 |

***Figure 6.5.*** Hazards of aspirin in a randomized trial of over nineteen thousand participants aged sixty-five years and older. Adapted from John McNeil et al., "Effect of aspirin on all-cause mortality in the healthy elderly," *New England Journal of Medicine* 379, no. 16 (October 2018): 1519–28, https://doi.org/10.1056/NEJMoa1803955. Description 33

Can diet significantly reduce the risk of cancer? Most of the studies in cancer are in experimental models (rather than in real people on certain diets); nevertheless, they demonstrate the increased risk from high-fructose corn syrup. They also indicate the *potential benefit* of methionine restriction, the advantage of caloric restriction, a ketogenic diet, low glycemic diets to limit tumor growth, and a dietary tryptophan metabolite released by a bacterium. A randomized trial of increased vegetable consumption versus a control diet in 478 men with early prostate cancer did not significantly affect progression. The foods that have the most unfavorable effects on inflammation markers were shown to be associated with a higher risk of colon cancer in a study of more than 120,000 people followed for twenty-six years. Foods that have been linked to lowering the risk of cancer include those with a rich source of isothiocyanates, such as broccoli, brussels sprouts, cauliflower; the lycopenes in tomatoes; high-fiber foods such as legumes and nuts (especially walnuts, which are high in ellagitannins); foods with high antioxidant and flavonoids like berries and pomegranates; and garlic, with its rich allicin content.

The latest estimate for the proportion of cancers attributable to modifiable risk factors is 40 percent, with the main factors shown in figure 6.6.

That compares to more than 80 percent of cardiovascular disease preventable via lifestyle factors.

There are now three drugs—tamoxifen, raloxifene, and anastrozole—that are FDA approved for prevention of breast cancer in women at moderate or high risk. These drugs block production or the effects of estrogen, but at least 15 percent of breast cancers do not have estrogen receptors. The "moderate to high" category of risk is determined by a breast risk calculator based on such rudimentary features as age, family history, age at first period, and age at firstborn child. Surely, we have far better ways to stratify risk of breast cancer, as I've reviewed in the section on mass screening with mammography.

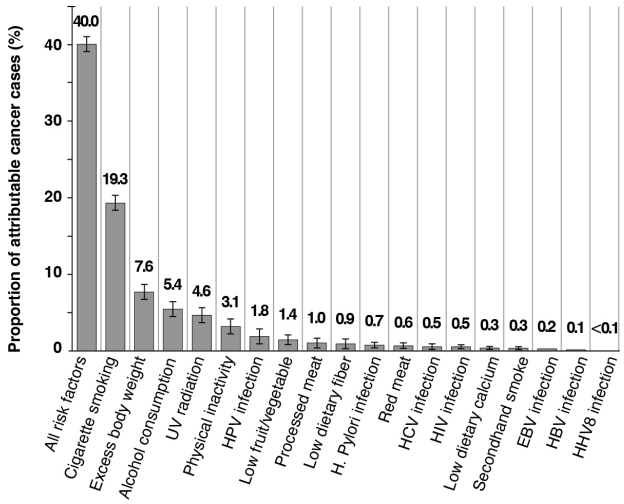



***Figure 6.6.*** Proportion of cancer cases with potentially modifiable risk factors. Adapted from Farhad Islami et al., "Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States, 2019," *CA: A Cancer Journal for Clinicians* (July 2024), https://doi.org/10.3322/caac.21858. Description 34

The story of the prevention for breast cancer has some common threads with the use of finasteride (versus placebo) for prevention of prostate cancer, a drug that lowers the levels of dihydrotestosterone. Way back in 2003, a randomized trial in nearly nineteen thousand men, aged fifty-five years and older, showed a 25 percent reduction over a seven-year follow-up period. Later, in analysis that linked the participants in that trial to their Medicare claims, which extended the follow-up period to sixteen years, and subsequently to the National Death Index, which produced a median follow-up of 18.4 years, there was persistent evidence of a 25 percent reduction, but the number of deaths was small (only ninety-eight in the nearly nineteen-thousand-men cohort) and no longer statistically significant. A systematic review of eight randomized trials found a 30 percent reduction for finasteride, but the prostate cancers in the finasteride group were of higher malignant grade. That may be due to the drug's marked lowering of prostate-specific antigen results, delaying the diagnosis. But this remains an unsettled issue, with no guidelines recommending finasteride to prevent prostate cancer.

Here again, we have ways to define the high risk of prostate cancer, such as multiancestry polygenic risk scores and many other layers of data, but we're not using them. It certainly remains possible that finasteride, let alone other drugs,

would provide important benefit for prevention if it were tested in a high-risk population rather than just using age as the sole criterion.

These examples provide marked contrast to successful cancer prevention when there is high specificity and efficacy, such as the benefits of human papilloma vaccination (HPV) to prevent cervical cancer, hepatitis B vaccination to prevent liver cancer, or prophylactic mastectomy surgery for people with BRCA mutations. Obviously, once we determine the infectious agent or the gene mutation widely, we are quite capable of moving on to a far more refined approach.

Why aren't we routinely assessing body-wide inflammation and building on the success of anti-inflammatory drugs such as the interleukin-1β drug that significantly reduced fatal cancers and lung cancer in a large randomized trial? Why aren't we aggressively tackling air pollution, which is unquestionably promoting cancer? We have a working blueprint for preventing so much cancer but ignoring many extraordinary opportunities in front of us.

While we need to revamp our approaches to cancer across the board—screening, accuracy of diagnosis, use of novel molecular diagnostics, treatment, and prevention—now, it will take years before we see the requisite updated changes in guidelines and routine medical practice for all things cancer related. At the population level, we cannot eke out many years of healthy aging without applying the remarkable progress we've made in our knowledge base. But this chapter, at the individual level, offers an anchor for your decisions, and your family members.

Part 2 has been about the big killers as measured by human lives lost. But when is a life lost exactly? What if we lose our minds in the second half of our lives?

# 7

# Neurodegeneration

In recent years, a revolution occurred in our understanding of immunity and the brain. For more than a century, the medical dogma was that the brain was "immune-privileged" in a separate compartment, isolated from and inaccessible to the body's immune system. But in the 1990s, initial evidence of T cells entering healthy brains was found.

That was a first step toward realizing that there is a brain lymphatic system (known as the glymphatic system) that communicates with the rest of the body, and that the skull bone marrow, meninges (membrane layers that cover the brain), and choroid plexus are an immune cell reservoir—a squad of immune cells that patrol the brain from its border, traversing in through and alongside vascular channels, cerebrospinal fluid, and specialized lymphatics. Triggered by synchronized brain cell firing, clearance of metabolic waste occurs through the glymphatic system plumbing—or, in the more colloquial term, brainwashing. Skull-meninges channels allow immune cells to flow back and forth without any need of blood vessels. In neurodegenerative diseases, the brain's immune cell system plays a major role with prominent involvement of CD4+ (helper), CD8+ (cytotoxic), NK cells, white blood cells, and monocytes. That role can range from helping to protect against degenerative changes, fighting brain cancer, to activating inflammation with pro-inflammatory effects.

Within the brain there is one type of cell that serves as a key player interacting with the immune response—the microglia, a specialized form of macrophage cells. As molecular pharmacologist Giulia Castellani wrote in a recent review in *Science*: "In adulthood, the microglia serve as the sentinels of the brain." They accumulate lipid droplets and release pro-inflammatory cytokines; they gradually lose their mobility and ability to perform their housekeeping function. This can all result in a disruption of the blood-brain barrier. Moreover, exhausted, "terminally inflammatory" microglia appear to contribute to the loss of synapses and the risk of neurodegenerative disease. Microglia interact with astrocyte cells, which can become active participants in neuroinflammation and are linked to brain cell death. Preventing neurodegenerative diseases is dependent, at least in part, on maintaining a healthy relationship between the brain tissue and immune system, with microglia serving as a gateway to their interaction.

There are several hallmarks of neurodegenerative diseases, including a buildup of misfolded proteins, inflammation, brain cell death, defects in DNA and RNA, and synaptic dysfunction.

Our brain structurally changes considerably as we age, even without exhibiting dysfunctional or degenerative features. In 2023, a large international collaboration, led by Richard Bethlehem and Jacob Seidlitz, reported a massive study of about 124,000 MRI scans from over 101,000 people, aged four months to one hundred years of age. This landmark paper enlightened the major dynamic structural changes as we age. White matter, gray matter, and cortical thickness (corresponding to the width of gray matter) are shrinking, whereas, in late adulthood, the volume of cerebrospinal fluid in the brain is sharply increasing. The exponential increase in ventricular volume, beginning at about forty years of age, is seen in figure 7.1.

The functional correlations for these structural changes are still being determined, but what we have now is a growth chart, just like what is routinely used in children, to be able to say what is normal or abnormal at different points over time. While these changes are at the macro level, there is single-cell and spatial omic characterization of the brain at the cellular and molecular levels.

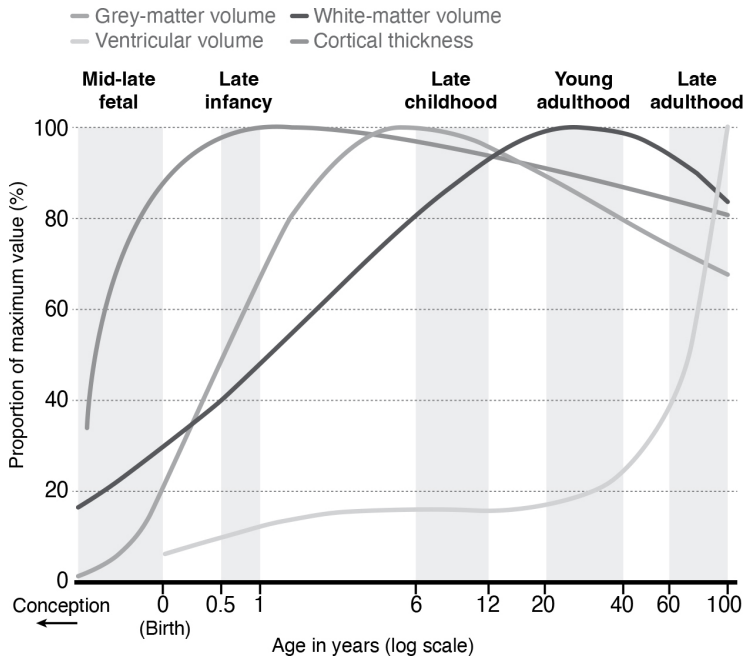



***Figure 7.1.*** Changes in brain anatomy as we age. Adapted from RAI Bethlehem et al., "Brain charts for the human lifespan," *Nature* 604, no. 7906 (April 2022): 525–33, https://doi.org/10.1038/s41586-022-04554-y. Description 35

Given those developments, let's delve into the two most widespread neurodegenerative diseases. We will spend most time on Alzheimer's because the confluence of new insights and medical technologies has had the most results there—so far.

## ALZHEIMER'S DISEASE

Two proteins with Greek letter designations—β-amyloid (Aβ) and Tau (T)—are center stage in the drama of Alzheimer's disease. When misfolded, they self-assemble to form Aβ plaques and neurofibrillary tangles (NFT), respectively, that propagate, disrupting neural circuits and brain cell function. In particular, the misfolding and aggregation of Aβ is the basis of the amyloid cascade hypothesis, the seminal disease-causing event that ultimately leads to Alzheimer's disease. More than three decades after it was conceived, it remains as a hypothesis, because the data supporting the central and unequivocal role of Aβ is mixed.

As seen in figure 7.2, this process evolves over decades. In the earliest stage, Aβ (secreted from cells as monomers, developing to fibrils) forms plaques that drive

immune cell activation. Tau pathology kicks in years later, which adds to loss of brain circuit synapses and function well before there is the onset of cognitive impairment.

Late-onset Alzheimer's disease accounts for 95 percent of the people who get the disease. It has high heritability between about 60 to 80 percent, although less than the 90 percent heritability of early onset (before age sixty-five years) Alzheimer's. The association of late-onset Alzheimer's with more than fifty genetic loci has enhanced our understanding of the underlying pathophysiology, which is not only related to β-amyloid and Tau proteins. A model that factors in all the known genetic pathways includes our immune response; how we handle cholesterol; our brain blood supply, which is affected by β-amyloid deposits; and how we remove proteins and engulf waste and toxic substances into our cells. Even more has been learned by single-cell sequencing approaches to blood vessel cells among others, reinforcing the role of many different genomic pathways.

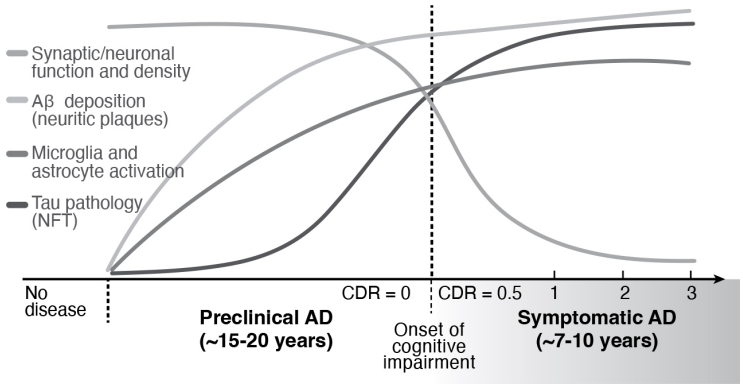



***Figure 7.2.*** Evolution of brain biologic abnormalities in the years before (and subsequent to) cognitive impairment and symptomatic Alzheimer's disease. NFT-neurofibrillary tangles. Adapted from Justin Long et al., "Alzheimer disease: An update on pathobiology and treatment strategies," *Cell* 179, no. 2 (October 2019): 312–39, https://doi.org/10.1016/j.cell.2019.09.001. Description 36

Knowing that it takes decades for the development of Alzheimer's disease in concert with its prelude of cognitive impairment, there has long been a search for some sign that the disease is coming, a biomarker. A few decades' warning would give us the opportunity to intervene and potentially halt the degenerative process. The potential for markers from the cerebrospinal fluid has been intensely explored but is of little practical value given the lack of appeal for people to undergo a lumbar puncture. A breakthrough in the field came with the discovery

of a plasma protein known as phosphorylated tau217 (p-tau217), and the ratio of phosphorylated to nonphosphorylated Tau, that was as good or superior to cerebrospinal fluid tests. The blood test for p-tau217 was more accurate, at a level of 91 percent, than cognitive tests and CT scans in people with memory problems. In a prospective study of more than twelve hundred patients, that compared favorably to primary care physicians (without the help of p-tau217), who had accuracy of only 61 percent of the time in their diagnosis, and neurologists 73 percent of the time.

Before p-tau217, we relied on positron emission tomography (PET) scan imaging fibrils of amyloid and Tau, which, in healthy people, predicts cognitive decline and Alzheimer's three to five years before symptoms are manifest. But acquiring these images is expensive, not widely available, and associated with substantial radiation exposure. Other plasma proteins have been demonstrated to be quite predictive, as seen in a study of more than fifty-two thousand UK Biobank participants followed for fourteen years, with assays for almost fifteen hundred plasma proteins (such as GFAP, GDF15, and NEFL). Still, the breakthrough with p-tau217 has provided us with what is still our best biomarker by far.

The commercial test availability of p-tau217 has engendered controversy for whether healthy people should be screened and undergo treatments that have recently been shown to have modest effects. The Alzheimer's Association advocates for screening and treatment, proposing that healthy people without symptoms but a positive p-tau217 should be classified as "Alzheimer's Stage 1." On the other side, cynics charge that it is simply a way for the medical industry to make money from people who aren't actually sick. Be that as it may, more than seven million Americans with mild cognitive impairment have not been diagnosed. Use of a simple blood biomarker is likely to greatly increase the number of diagnoses from the current level of approximately 8 percent of people aged sixty years and older. If we extrapolate from previous studies of cognitively normal people, almost 16 percent of people aged sixty and 23 percent aged seventy would test positive for mild cognitive impairment. That's a huge number of people at risk.

Still, my view in the face of the raging debate is that this test should not be done routinely in people who are cognitively intact, nor should it be used to assign a diagnosis of pre-Alzheimer's. That is not warranted or appropriate based on what we know. However, this biomarker has considerable utility as part of a comprehensive risk assessment. It is a key component for how Alzheimer's disease —at the individual level—may be prevented or significantly forestalled in the future.

## THE APOE GENES

For over thirty years, we've known about one group of people who carry high risk of Alzheimer's due to their apolipoprotein E (APOE) genotype. The APOE4 allele is the strongest genetic risk factor: two copies, which are present in only 2 percent of the Caucasian population (the prevalence varies widely by ancestry and ethnicity) and denote a fifteenfold higher risk in people of European ancestry (less in African ancestry). About a fourth of the population are APOE4 carriers with one copy and have about a threefold risk. On the other hand, the APOE2 allele lowers the risk, and APOE3 is neutral. The Mayo Clinic Study of Aging followed almost five thousand people, age fifty-five to ninety-two years, in Olmsted County, Minnesota, for 9.4 years. For carriers of APOE4 who had high PET imaging amyloid levels at age sixty-five, the lifetime risk of Alzheimer's was 75 percent of women and 62 percent of men. In non-APOE4 individuals, that risk was reduced to 53 percent for both sexes. There appears to be sex-specific alterations in APOE4 carriers, with females more prone to white blood cell–microglia interaction that promotes amyloid plaque formation.

APOE4 hurts the brain in various ways, but an important one is the way it causes faulty cholesterol metabolism, which in turn increases cholesterol buildup in cells, impairs myelination (the critical insulation around nerve fibers), and renders the blood-brain barrier dysfunctional.

From a study of over five hundred people with two copies of APOE4, we saw almost all of them manifest Alzheimer's pathology. Also, the onset of symptoms and biomarker changes, earlier than in individuals with other APOE genotypes, was more predictable. However, many individuals are resilient and do not

develop symptoms or cognitive decline. Nonetheless, the findings were considered to redefine having two copies of the APOE4 gene as a distinct genetic form of Alzheimer's disease. Also, apart from other APOE genotypes, single-cell sequencing of brain microglia in patients who died with Alzheimer's disease with two copies of APOE4 revealed liquid droplets that were neurotoxic, promoting Tau pathology.

So it is good news that removal of APOE4 in the experimental model protects against the loss of myelination, inflammation, and impact of Tau. That's also the target and goal of our current amyloid antibody therapies. Gene therapy in patients directed at removing APOE4 is already being tested in patients.

Further good news is that about 30 percent of people with heavy amyloid and Tau deposits in their brain do not ever manifest Alzheimer's disease clinically. That's not just because they don't live long enough; it may be attributable, at least in part, to biologic modifying factors. While we cannot predict that favorable outcome yet, we are learning more about genes that protect and promote brain resilience to these protein deposits, besides APOE2, such as MEF2, APOE Christchurch, Klotho, fibronectin 1, Reelin-COLBOS, and APOE-R136S. For example, a gene variant that promotes amyloid accumulation around brain blood vessels but not Alzheimer's disease pathology has been identified. Add to that epigenetic changes, as tracked by DNA methylation, that foster resilience. Ironically, there may also be some cognitive benefits of the APOE4 allele with respect to boosting short-term memory.

I must emphasize that genetic risk for late-onset Alzheimer's extends well beyond APOE4. A polygenic risk score based on hundreds of genomic variants can accurately predict a doubling of the risk of developing Alzheimer's. Notably, more than half of these risk genome variants involve the innate immune system, further framing Alzheimer's as a neuro-inflammatory condition. The polygenic risk score complements the blood biomarker as a way to identify high-risk individuals, so if there is an effective intervention, we can prevent the disease from ever manifesting or significantly delay its presentation.

Once a person has reached an advanced age, a family history of Alzheimer's is the most important risk factor for late-onset Alzheimer's disease. The pattern of parental transmission to offspring by mothers, compared with fathers, is

different. In a study with more than forty-four hundred participants with PET scans for β-amyloid, maternal history of memory impairment at any age was associated with increased β-amyloid in offspring, whereas for fathers that was only the case for early onset memory difficulties. The reason for this remains unclear; the potential role of transmission for the maternal X chromosome or mitochondria has been speculated.

The use of deep-learning AI of the MRI has been shown to improve accuracy of diagnosis of Alzheimer's. A multimodal AI model performed exceptionally well for accurately classifying ten different types of dementia, exceeding neurologists. AI has even been able to help estimate how long until symptoms manifest based on abnormal imaging, genomics, and clinical data. That feeds into our *infra* potential prevention strategy.

## LIFESTYLE+ FACTORS

For prevention or delaying the onset, we do know lifestyle+ factors play a vital role in our overall health. We've reviewed many of these, since the benefits of healthy lifestyle transcend the cardiovascular system and extend to promoting brain health. But it is worth emphasizing the difference that can be made to the onset of neurodegeneration.

Walking matters! To determine the relationship between steps and all-cause dementia, more than seventy-eight thousand UK Biobank participants with wrist accelerometers were followed for seven years: a daily dose up to ninety-eight hundred steps was linked to protection. There are nearly two thousand reports and eighty randomized trials that have studied the relationship between physical exercise and cognition. Overall, there is a consistent, though small, favorable effect of exercise for cognition. Some new studies have probed the mechanism for this benefit, such as improved clearance of Aβ thanks to our brain plumbing discussed above. In an animal model of aging, exercise rejuvenates microglia cells and reduces brain inflammation. Interestingly, an extensive gene expression analysis of over one thousand genes linked to Alzheimer's identified "exercise as the top theoretical treatment."

The Mediterranean-DASH Intervention for Neurodegenerative Delay diet randomized trial did not show benefit in 604 participants with a family history of Alzheimer's compared with a control diet with mild caloric restriction. However, a prior double-blind randomized trial conducted in Finland with 1,260 participants, testing an intervention that included the Mediterranean diet, aerobic and strength training exercise, and cognitive training showed marked improvement to complex memory, executive functioning, and mental processing speed. In contrast, I've previously highlighted the risk of a diet high in ultra-processed foods for dementia.

Those results are aligned with a longitudinal study that assessed a healthy lifestyle with the MIND diet, exercise, and limiting alcohol consumption. Among 586 participants, a higher lifestyle score meant better global cognition. Obesity and abdominal fat were seen to increase the risk of Alzheimer's. Other small clinical trials reinforce the contribution of lifestyle factors. In an eight-week randomized trial of intermittent fasting in cognitively intact older adults brain aging MRI metrics improved similarly as a control, nonfasting, healthy diet. A small randomized trial of a comprehensive lifestyle strategy in older adults with mild cognitive impairment showed benefit of cognitive function testing and brain biomarkers.

The importance of sleep for brain health is well established. As we've discussed, it is during sleep that our metabolic waste products are cleared through cerebrospinal fluid and the glymphatic system. Even a single sleepless night in young individuals has demonstrated accumulation of Aβ. A UK Biobank study in over 150,000 participants, 48,000 of whom underwent brain imaging, with cognitive testing and six- to ten-year follow-up, indicated that seven hours of sleep linked peak cognitive performance and preserved brain structure. Multiple studies in midlife (fig. 7.3) and older adults indicate that sleep disturbance is a risk for late-onset dementia. Note again the seven-hour cutoff for association with the lowest hazard ratio. It's a surprising result. After seven hours, more sleep is associated with more risk.

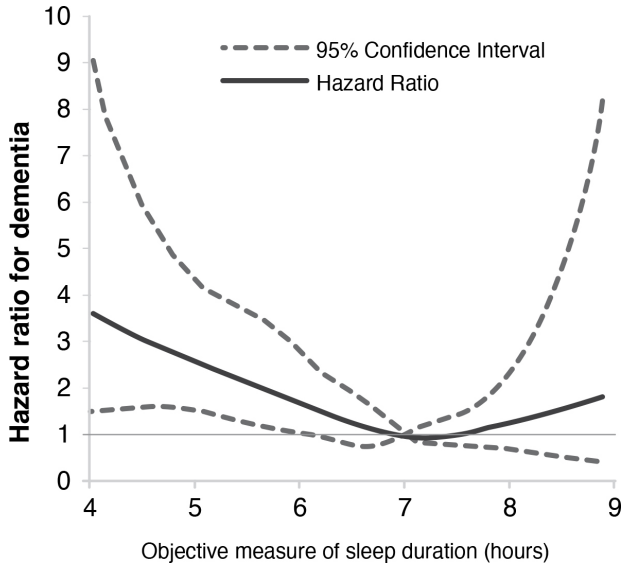



***Figure 7.3.*** The relationship of sleep duration and risk of dementia. Adapted from Severine Sabia et al., "Association of sleep duration in middle and old age with incidence of dementia," *Nature Communications* 12, no. 1 (April 2021): 2289, https://doi.org/10.1038/s41467-021-22354-2. Description 37

Other cardiovascular factors are tied to risk of Alzheimer's and all-cause dementia, including a very high HDL, high LDL, and elevation of systolic blood pressure. The quality and quantity of daily social encounters have exhibited a strong inverse association with risk of Alzheimer's. In people at high risk for Alzheimer's, a randomized trial subgroup analysis showed the benefit of audiological counseling and hearing aids to reduce three-year cognitive decline. From the United Kingdom Ageing Cohort, the relationship between musicality and cognitive function was highlighted. Playing an instrument and singing were associated with preserved memory and executive function.

The role of the environment is receiving more attention for increasing the risk of Alzheimer's, with multiple studies reinforcing the impact of air pollution. At autopsy of 224 participants with Alzheimer's disease, traffic-related particulate matter correlated with a near doubling of risk, prominently among people without the APOE4 alleles. Using explainable AI, a deep dive of the relationship between air pollution and Alzheimer's in Italy revealed that ozone and nitrogen dioxide were the main pollutant contributors. Beyond air pollution, on the basis of epigenetic changes, concerns have been raised on various metals (cadmium, arsenic, lead, aluminum, mercury, nickel, and manganese), aflatoxin B1 found in some foods, endocrine-disrupting chemicals (bisphenol A and polybrominated

biphenyls), pesticides, alcohol, and tobacco. The concern over the pervasive presence of microplastics and nanoplastics, which we inhale and ingest, and which can circulate in our bloodstream, has recently been amplified by finding these in our brains. These plastics have an alarming capacity to incite inflammation there. Pervasive environmental chemicals, such as the quaternary chemical compounds found in disinfection agents and personal care products, or organophosphate flame retardants in furniture and electronics, have been shown to impair oligodendrocyte cell development. An extensive study of the modifiable factors associated with brain aging showed that diabetes, pollution, and alcohol intake were the most deleterious risk factors. The 2024 Lancet Commission Report on Dementia, which added untreated vision loss and high LDL cholesterol as modifiable risk factors, estimated that 45 percent of dementia can be prevented or significantly delayed. Based on the data reviewed here on sleep health, unprocessed food intake, and risk after severe COVID in older adults, that raises the number of factors to seventeen, so perhaps up to half of the burden of dementia can be substantially reduced with attention to these risks (fig. 7.4).

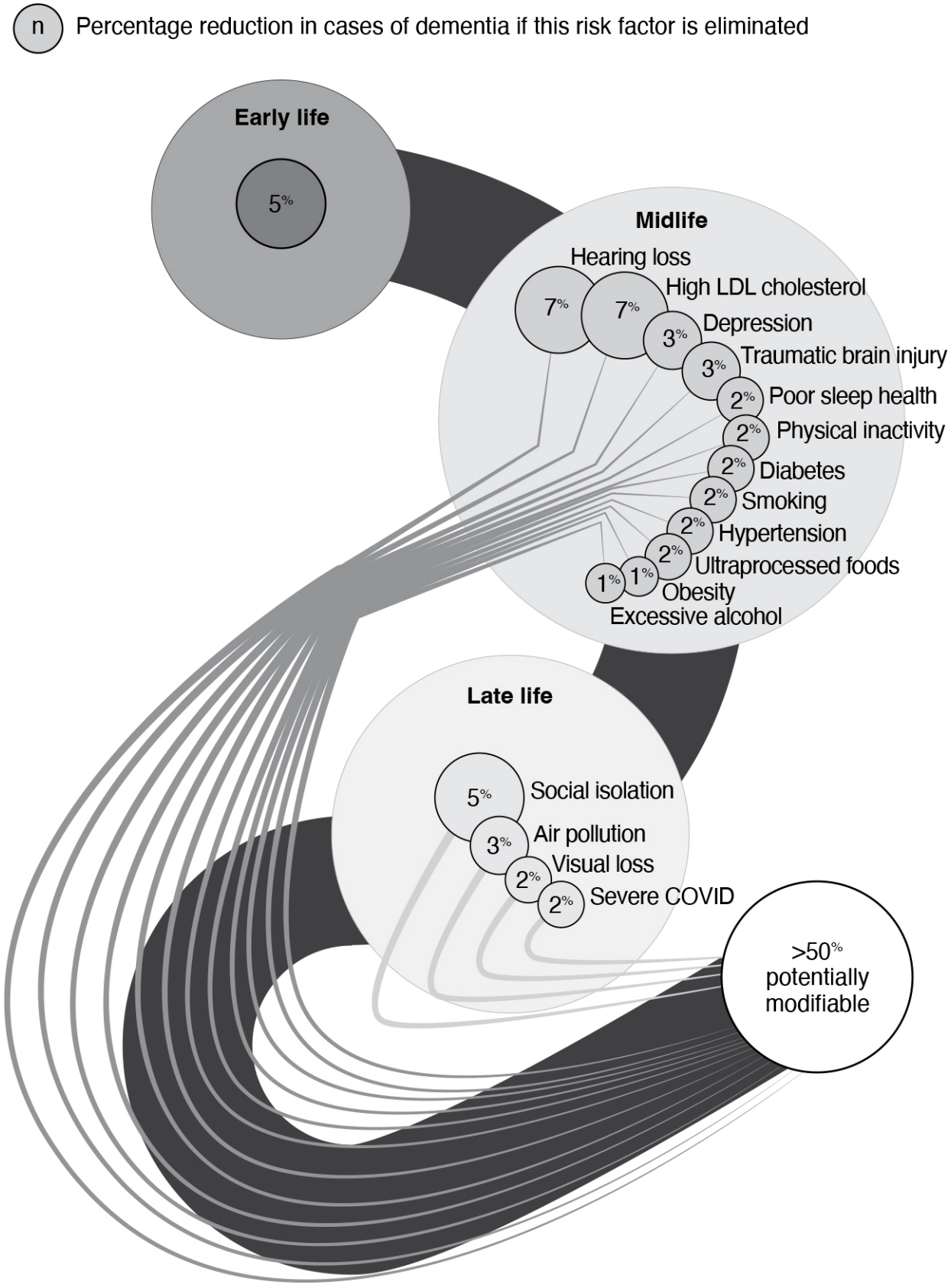

n
Percentage reduction in cases of dementia if this risk factor is eliminated
Early life
5%
Midlife
Hearing loss
7%
High LDL cholesterol
7%
Depression
3%
Traumatic brain injury
3%
Poor sleep health
2%
Physical inactivity
2%
Diabetes
2%
Smoking
2%
Hypertension
2%
Ultraprocessed foods
2%
Obesity
1%
Excessive alcohol
1%
Late life
Social isolation
5%
Air pollution
3%
Visual loss
2%
Severe COVID
2%
>50%
potentially
modifiable

***Figure 7.4.*** Modifiable risk factors for dementia. Adapted from Gill Livingston et al., "Dementia prevention, intervention, and care: 2024 report of the Lancet Commission," *Lancet* 404, no. 10452 (August 2024): 572–628, https://doi.org/10.1016/s0140-6736(24)01296-0. Description 38

## THE GUT MICROBIOME

We can see the relationship between the gut microbiome and Alzheimer's disease getting clearer. In a 2023 study from Washington University in St. Louis comparing 49 individuals with preclinical high risk of the disease to 115 participants without such high-risk biomarkers, the gut microbiome offered intriguing insights. Not only did the microbiome ingredients differentiate the two groups, but in examining brain PET scans, reinforcing correlations were found in the Aβ plaques and Tau, along with cerebrospinal fluid p-tau-181 level and the ratio of Aβ42/Aβ40. So, what was causing what?

While that study reinforced a correlation, gut microbiome transplantation from people with Alzheimer's and aged-matched healthy controls to young healthy rats is even more revealing. In that animal model, as assessed by a team of scientists at University College Cork, Ireland, there was cognitive impairment proportional to the severity of the dementia, and abnormal behavior tied to changes in the hippocampus metabolome. The researchers also found diminished ability to grow new neurons in the lab from the blood of the participants with Alzheimer's. Multiple other studies in mouse experimental models of Alzheimer's have confirmed gut microbiome–induced microglial activation, underlying the gut's role of promoting Aβ plaque and hyperphosphorylated Tau. In particular, the metabolites from the gut microbiome, which include short-chain fatty acids, activate immune cells to produce cytokines that disrupt the blood-brain barrier and induce neuroinflammation. Immune cells from the gut have even been found to migrate to the brain. We must conclude that the gut-brain axis plays a pivotal role in the development of Alzheimer's disease. That also provides an opportunity to intervene with the rapidly evolving landscape of prebiotics and probiotics in the future, manipulating the gut microbiome's composition to reduce brain inflammation.

## TREATMENTS THAT DON'T SEEM TO WORK

The graveyard of putative Alzheimer's disease treatments aimed at β-amyloid and other pathways is replete with hundreds of failed attempts over the past three decades. That appeared to change in 2016, when a paper claimed that aducanumab, a monoclonal antibody, reduced brain Aβ plaque after one year of monthly infusions; it claimed "compelling support for the amyloid hypothesis" if confirmed in ongoing phase 3 randomized clinical trials. Two phase 3 trials followed but were stopped early in 2019 because the treatment showed no clinical benefit to participants with mild cognitive impairment or mild Alzheimer's. The trials were later restarted by the biotech company Biogen, who then claimed PET imaging showed that the high dose in one trial effectively lowered β-amyloid levels. Defying all ten experts on the FDA Advisory Committee who recommended against approving the drug, the FDA went ahead and gave this antibody, with the trade name Aduhelm, a green light in 2021. Some members of the FDA Advisory Committee resigned in protest, one proclaiming it as "probably the worst drug approval decision in recent U.S. history." Serious side effects such as brain swelling in 35 percent of participants and microhemorrhages in 20 percent had not swayed the decision. It was later learned by STAT news that the FDA was working hand in hand with the company to help resurrect the drug. After approval, with a list price of $56,000 per year, Aduhelm was scantly prescribed, it did not get Medicare reimbursement, and Biogen gave away its ownership of the drug to Neurimmune in 2024. All in all, not a good start for the first amyloid monoclonal antibody that seemed to reduce brain plaque.

The monoclonal antibody entry that next reported positive results was lecanemab (Leqembi), with a phase 3 trial published in 2023. Compared with placebo, intravenous antibody infusions every two weeks for eighteen months led to reduction of β-amyloid and "moderately less decline" of cognition. Amyloid-related imaging abnormalities, brain edema, and microhemorrhages occurred in 12.6 percent of the antibody group and 1.7 percent in the placebo group. In 3 percent of the antibody group, these three factors caused serious problems, requiring urgent evaluation and management, compared with none receiving placebo. In the treatment group, the three factors were proportionally worse for

individuals with the APOE4 gene than noncarriers. There was also more brain shrinkage with the antibody and a 3 percent incidence of major bleeding in people taking blood thinners. Despite these bad results, Leqembi was approved by the FDA in 2023 with an annual cost of $26,500 per year, and the associated imaging and monitoring brings up the cost to over $75,000 a year.

The third antibody drug to come along with signs of efficacy in a phase 3 trial was donanemab. The researchers used the same enrollment criteria of mild cognitive impairment or early Alzheimer's, but with a monthly rather than biweekly infusion over eighteen months and stopping the treatment at twenty-four or fifty-two weeks if the PET scan showed sufficient amyloid clearance. For analysis, the trial partitioned the participants by level of Tau accumulation. Donanemab achieved more reduction of amyloid than lecanemab but also induced almost twice as many bad effects. Along with more edema and microhemorrhages, three deaths in the trial were from donanemab treatment. These fatal events may be related to accumulation of amyloid around brain blood vessels. Treatment appeared to delay the deterioration of cognitive function for four months, more for the group of patients with low to medium Tau levels on PET imaging. One difference from the lecanemab trials is that donanemab treatment was stopped when the brain scan showed minimal to no amyloid. This occurred in two-thirds of participants after one year of treatment. In contrast to the treatment's impact on amyloid by brain imaging, using standard cognitive ability criteria, the reduction was 0.7 points compared with 0.5 points with lecanemab, a modest indication of incremental benefit. However, a full one-point reduction has been deemed to signal a clinically meaningful outcome, so this is still somewhat short of that goal. Nevertheless, donanemab was approved by the FDA in 2024.

So far, we see two monoclonal antibodies directed to β-amyloid clearing plaque (up to 80 percent with donanemab), a delay of a few months in cognitive decline, but with significant risks, accelerated brain shrinkage, and high cost. The dissociation between the marked reduction of brain amyloid but only modest clinical improvement matters. Moreover, the treatment strategy is highly complex, even with use of a blood biomarker such as p-tau217. The potential qualifying patient must undergo PET and MRI imaging; come to a specialized

clinic for the antibody infusion every two or four weeks; have frequent MRIs before fifth, seventh, and fourteenth infusion (the FDA recommendation for lecanemab); and undergo follow-up PET imaging.

The participants in these trials were about 90 percent white, so we have insufficient evidence for other races and ethnicities. The evidence for the two monoclonal antibodies that were deemed positive is flanked by an extraordinary series of failures of other β-amyloid antibodies, such as crenezumab, bapineuzumab, gantenerumab, solanezumab, and others. It was particularly disconcerting to see the lack of benefit in people who were asymptomatic but carried a gene mutation for Alzheimer's.

Speculation abounds as to why two of so many different antibodies seemed to achieve a small clinical benefit. Each of these binds to different parts (epitopes) of the Aβ-42 protein. Their binding strength to β-amyloid varies, with donanemab being the strongest. In any case, the anti-amyloid approach is on fallow ground, despite the past FDA support.

The field has moved to testing the antibodies in people who are asymptomatic but with biomarker-based risk (such as p-tau217) in multiple clinical trials. The idea is that the risk will be decreased and the benefit augmented before there has been accumulation of Tau and more advanced pathological changes in the brain. Another direction is to test multiprong therapies, such as targeting β-amyloid, Tau, and the gliosis-linked brain inflammation. Tau-directed monoclonal antibodies, antisense oligonucleotides, and vaccines are being pursued in clinical trials.

A pill treatment that has raised new hopes of success modulates the p75 neurotrophin receptor, a potent molecular switch for neuron survival and synapse integrity. In a randomized trial of over 240 participants who received placebo or two different doses of a p75 modulator, twice daily, there were no safety concerns, and, in a prespecified subgroup, the twenty-six-week treatment led to about 50 percent slowing of cognitive decline, along with favorable impact on cerebrospinal fluid biomarkers and brain imaging. We await the results of large-scale testing, but as a pill, without risk of brain edema and hemorrhages, this intervention would be highly advantageous compared with the anti-amyloid antibodies.

## OTHER POTENTIAL THERAPIES

Lexeo Therapeutics has moved forward with gene therapy for participants with two copies of APOE4. Participants received a copy of APOE2, the allele that carries a decreased risk of Alzheimer's, which was attached to a virus vector and delivered directly into their cerebrospinal fluid via lumbar puncture. Subsequently, appearance of APOE2 was detected in their cerebrospinal fluid with lowering of Tau levels, and without serious side effects. Gene therapy (adding a gene) is quite different from genome editing (modifying a gene). The success in CRISPR genome editing for sickle cell disease, β-thalassemia, and other conditions has led to introducing the rare, protective APOE variant (APOE3-R136S, Christchurch) in the mouse model of Alzheimer's providing protection, especially notable with two APOE4 copies. Other editing targets, such as the amyloid precursor protein (APP) and presenilin-1 (PSEN1), have been approached in experimental models and cells, respectively.

Quite intriguing is a nasally delivered Tau monoclonal antibody. A single dose had a marked impact in the aged mouse tauopathy model, clearing these pathologic Tau aggregates from inside cells and improving cognitive function. These antibodies have been shown to recognize Tau in human brain tissue. Hopefully this innovation, far simpler than the current monoclonal antibody administration requiring frequent intravenous infusion, will soon move into clinical testing.

Conceptually, rejuvenation of the aged immune system (immunosenescence) to prevent Alzheimer's disease has appeal. Proof of concept was shown in an aged mouse study with bone marrow transplantation from young mice, which substantially reduced β-amyloid burden, degenerative changes of neurons, and brain inflammation, and improved behavioral deficits. Activation of microglia, resident immune cells in the brain, would also be expected to protect against age-related brain pathologies. We'll come back to these prospects for immune system intervention later.

The use of MRI-guided focused ultrasound to open the blood-brain barrier in combination with β-amyloid monoclonal antibodies has been tried in a limited number of patients. With just three patients treated, this became the subject of

extensive coverage by the *60 Minutes* television news show. With such a small number of patients treated, without controls, and lack of clear-cut clinical benefit, these came across as a sensationalized report. Other strategies are being tested to open the blood-brain barrier to promote the drug treatments.

In a small randomized trial of fifty participants with mild to moderate Alzheimer's, repetitive transcranial magnetic stimulation appeared to modestly slow cognitive decline while the sham control group had worsening of their results. A headset that uses gamma frequency light and sound stimulation has preliminary data for slowing decline of cognitive function and preventing brain atrophy, and is now completing a larger trial for potential FDA approval.

Repurposing drugs to help treat Alzheimer's has also been pursued, such as potential benefit of intranasal insulin for improving memory function, Viagra with a link to possible reduction of risk, and a loop diuretic water pill. None of these treatments has yet been validated by randomized trials.

A provocative study has raised the potential of treating "jumping genes," known as retrotransposons. By transforming skin cells to pluripotent stem cells and onto neurons, from patients with Alzheimer's disease and controls, many cardinal features of the disease (such as Aβ deposition and Tau accumulation) were reproduced in organoid 3D cell culture and reversed by lamivudine, a drug that stabilizes transposons.

To summarize what we've learned in recent years, the evidence of net clinical benefit for two monoclonal antibodies is modest at best. Even getting rid of all the amyloid deposits in the brain may prove to be too late to meaningfully interrupt the inexorable progression of the condition. This pertains to treatment for people who are already affected with mild cognitive impairment of early Alzheimer's disease. That's very different from achieving prevention or marked delay of the process in people at high risk.

## PREVENTION

The breakthrough in this field has been a simple blood biomarker that indicates high risk and lays the foundation for the exciting possibility of primary prevention. With all the related knowledge we've accrued, I consider this the

exemplar for changing the natural history of a devastating age-related disease. Remember the propitious advantage on our side: we know it takes more than two decades to develop this condition. A simple blood test of inflammation, C-reactive protein, in early adulthood, assessed serially on more than two thousand participants over eighteen years, partitioned the risk of cognitive decline. Let me outline the approach that relies on identifying people at high risk, close surveillance, multimodal AI analytics of the data, and bespoke interventions.

I've written elsewhere about the potential for medical forecasting to simulate the remarkable success of AI-powered GraphCast weather forecasting (with 99.7 percent accuracy!), which is truly amazing. In figure 7.5, we see ways to partition high-risk individuals integrating multiple layers of data. That includes not only known clinical factors such as hearing loss, osteoporosis in women, erectile dysfunction or prostatic hypertrophy in men, and paternal history, but also lifestyle+ factors that we've just reviewed. Complementing this are the APOE genotype, a polygenic risk score, a blood p-tau-217 test, inflammation blood markers such as hs-CRP, a brain organ clock cluster of plasma proteins, a gut microbiome for its constituents and presence of certain pro-inflammatory microbes, and overall body-wide epigenetic age by DNA methylation. A retina image is independently helpful for prediction of high risk. Speech, analyzed with AI methods, can predict Alzheimer's disease within six years. Multimodal AI is used to quantitatively determine the risk level, factoring in all these data inputs, and has even displayed accuracy with more limited data. A threshold can be selected for categorization of high risk, such as top 5 or 10 percent of individuals assessed.

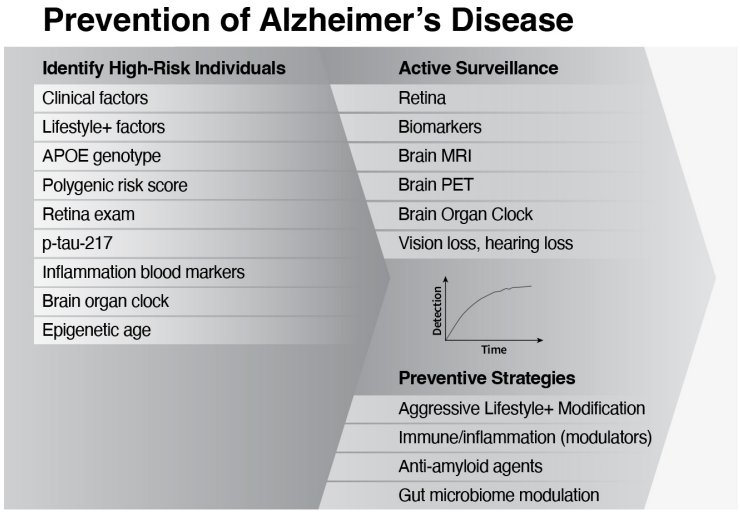



***Figure 7.5.*** Prevention of Alzheimer's disease. A multimodal AI comprehensive assessment of risk to identify high-risk individuals, followed by active surveillance and aggressive preventive strategies. Description 39

People deemed at high risk must undergo further assessment and close surveillance. This includes a conventional brain MRI, or a resting-state functional MRI that can further differentiate risk and predict the disease up to nine years in advance. PET imaging, as used in the clinical trials, quantifies the levels of amyloid and Tau. Imaging can be done on a serial basis over time to determine whether there is progression and its rate. Add to this serial retina, brain organ clock, inflammatory marker assessments. Brain age, determined by AI of combined MRI and PET imaging, can be compared with chronological age and may prove useful as a means of tracking progression.

Multimodal AI of all these layers of data, indexed by time, can be used to establish the tempo of an individual's condition, just as it has been used to accurately diagnose the many different types of dementia. It has also been used to predict the time frame of progression in people with mild cognitive impairment and evidence of β-amyloid accumulation. This could lead to aggressive interventions for lifestyle+ factors, use of anti-inflammatory drugs that are currently in clinical trials (such as GLP-1, or p75 drugs) or are demonstrating considerable potential, and, if need be, anti-amyloid treatment. This comprehensive approach is not yet validated but is worthy of rigorous assessment now.

Accurate definition of high risk would be critical for vaccines to protect against Alzheimer's disease. There are multiple ongoing trials, such as AC Immune in Switzerland with two Alzheimer's vaccines, one against β-amyloid and the other against Tau, or Vaxxinity in Florida, launching a clinical trial in three thousand participants with its amyloid directed vaccine. Six clinical trials are in progress in China, with vaccines directed to β-amyloid or Tau.

Meanwhile, as we await vaccines directed against Alzheimer's disease, more evidence of an association of the recombinant shingles vaccine (Shingrix) with reduction of dementia, including Alzheimer's, has emerged, which also raises the potential role of herpes zoster virus. Generally recommended for all people aged fifty years and older, this would be another specific consideration for high-risk individuals. Along with other reports, the potential tie-in of infections and Alzheimer's is getting increasing attention. The possibility that Alzheimer's may be caused by latent viruses, such as herpes or cytomegalovirus, has led to clinical trials with antiviral drugs.

## PARKINSON'S DISEASE

Second to Alzheimer's disease on the list of neurodegenerative disorders, Parkinson's is on a steep rise of prevalence expected to affect as many as seventeen million people by 2040. More than two centuries since James Parkinson's classic *An Essay on Shaking Palsy*, its pathogenesis remains unclear, and there are no validated disease-modifying treatments that stop or delay its progression. Age is the primary risk factor for both Parkinson's and Alzheimer's diseases. As is the case with Alzheimer's, it takes decades for the underlying disease process to develop, begging for us to get a head start to interrupt it from occurring.

As Alzheimer's is substantially driven by β-amyloid accumulation, so Parkinson's is driven by α-synuclein protein accumulation. There are many routes to a surfeit of α-synuclein, including mutations in genes, the most common one being in SNCA, which encodes for the synuclein protein. Mitochondrial dysfunction, impaired waste disposal, immune activation, and cell-to-cell spread of the toxic protein also contribute to the problem. Whatever the route, the result

is microglial activation, activation of the innate and adaptive immune response, substantial neuroinflammation, dysfunction of synapses, and loss of neurons.

Single-cell sequencing investigating the loss of neurons related to the neurotransmitter dopamine in a part of the midbrain (the substantia nigra) identified a family of affected cells that appear critical to the progression of the disease. Single-nuclei sequencing raised the potential that not just microglia but also a subtype of oligodendrocytes have a role. Such zooming in on cells and nuclei generates new insights for how Parkinson's takes hold in the brain.

But Parkinson's disease is not only a brain disorder. It is one of chronic inflammation that especially affects the gastrointestinal tract, with symptoms of difficulty in swallowing, constipation, delayed gastric emptying, and irritable bowel syndrome, which are concurrent or may even precede the diagnosis of Parkinson's. The gut symptoms can precede the Parkinson's motor symptoms by decades. Substantial crosstalk occurs between the gut and brain, with potential spread of α-synuclein from the gut via the vagus nerve to the brain. A recent finding that patients with inflammatory bowel disease produce α-synuclein lent further credence to Braak's hypothesis that the disease can or does originate in the gastrointestinal tract's enteric nervous system.

There is a substantial genetic component to Parkinson's with mutations in autosomal dominant genes (LRRK2 is the most common, also SNCA and VPS35) and autosomal recessive (PRKN, PIKN1, DJ1). Polygenic risk based on nearly 90 genome variants accounted for up to 36 percent of the heritability of the disease. Separately, a polygenic risk score for cognitive impairment that can occur in Parkinson's has been developed. A treatment someday to preserve mitochondrial function may come from the recent discovery of a protective mitochondrial gene variant (promising about 50% less risk of developing Parkinson's). As might be anticipated, individuals with an APOE4 allele, who also develop Parkinson's, have an increased risk of dementia.

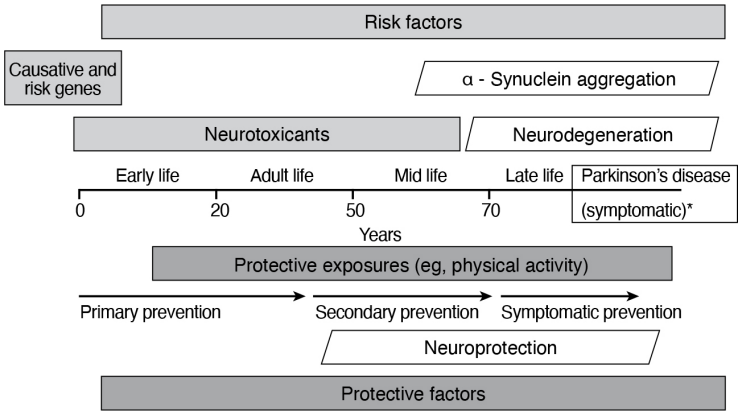



***Figure 7.6.*** Risk and protective factors for development of Parkinson's disease. Adapted from Yoav Ben-Shlomo et al., "The epidemiology of Parkinson's disease," *Lancet* 403, no. 10423 (January 2024): 283–92, https://doi.org/10.1016/S0140-6736(23)01419-8. Description 40

The risk of developing Parkinson's (fig. 7.6) increases as we age, and with exposure to neurotoxins such as pesticides, air pollution, or microplastics, and lower socioeconomic status. Physical activity, caffeine, and—would you believe?—cigarette smoking appear to reduce the risk. I don't recommend taking up the latter. Diets with high vegetables, fruits, and grains are linked with lower risk, while high fat and diets high in ultra-processed foods are associated with increased risk.

The hunt for a biomarker for Parkinson's has lagged the progress for Alzheimer's disease, but recent work is very encouraging for α-synuclein seed amplification assays (SAA) from cerebrospinal fluid for identifying individuals with high sensitivity and specificity. This was validated in a large study of more than 1,100 participants, which included 545 with Parkinson's, 51 people with the precursor signs of loss of smell or rapid eye movement sleep behavior disorder (RBD), and 310 carriers without manifesting Parkinson's. Being able to differentiate carriers of gene mutations who do not manifest the disease with this biomarker presents a real potential advantage. Alternative to the need for a spinal tap, the SAA assay from skin or skin biopsy for phosphorylated α-synuclein has high accuracy. Multiple types of PET imaging (dopaminergic, metabolic, or cardiac denervation) have been shown to have high sensitivity, but these are not widely available, they are expensive, and they expose the individual to high doses of radiation.

We're still in need of a reliable blood test, and many candidates have been raised, such as glycoprotein nonmetastatic melanoma protein B (GPNMB), a mitochondrial damage assay, DOPA decarboxylase for dopaminergic cell loss, and many others. A simple, noninvasive biomarker that can be used to accurately predict Parkinson's years before it is clinically declared is a major unmet need. That's why the report of a blood panel of over 120 proteins that could predict Parkinson's disease up to seven years before symptom onset is seen as a very encouraging advance. Several more are in the works.

We've had better forecasting success with the retina image. In two studies in which I collaborated with Pearse Keane and his colleagues at Moorfields Eye Hospital and the University College of London in the United Kingdom, AI analysis of retinal images could accurately predict Parkinson's' disease up to nine years before symptoms occurred. As emphasized with Alzheimer's disease, the retina gateway to the brain, with the help of the digital eyes of AI, is providing an important means for differentiating high risk for neurodegenerative disorders.

Detection and tracking the progression of the disease are having success with wristband accelerometers that can pick up change in gait and motor activity years before symptoms, nocturnal breathing signals from radio waves, and multimodal AI of imaging, electronic health records, and genetic and metabolomics to predict an individual's trajectory and treatment response.

The current treatments for Parkinson's are diverse and are all symptom directed, including a long list of medications for treating the motor abnormalities and the neuropsychiatric sequelae of depression, anxiety, sleep disturbances, hallucinations, orthostatic hypotension, urinary dysfunction, and dementia. Brain surgery to enable deep brain stimulation or an ablative procedure without implantation of hardware are other options.

For the first time, we may be on the brink of therapy to slow or stop the progression of Parkinson's disease. Prasinezumab is a monoclonal antibody that binds aggregated α-synuclein and was assessed in a randomized trial of Parkinson's disease. In the subgroup who were taking monoamine oxide inhibitors or had other prespecified signs of faster progression, there was evidence for slowing of the motor symptom (tremor, stiffness, slowness of movement) progression over four years of follow-up.

Even more encouraging are the consistent findings from three randomized trials of our new friends the GLP-1 drugs. Lixisenatide compared with placebo stopped progression of the motor signs for people with early Parkinson's. Exenatide showed significant benefit for motor symptom score in a smaller placebo-controlled trial. Another randomized trial of exenatide with longer follow-up found similar benefit with the conclusion, by Thomas Foltynie of University College of London and coauthors: "There is a strong indication that GLP-1 receptor agonists may have a useful role in future treatment of Parkinson's disease." In these trials, the benefit endured after the GLP-1 drugs stopped, and the advantage may further widen during extended follow-up.

Although not as palatable or practical as a drug treatment, fecal microbial transplant from healthy donors has been assessed in a small randomized trial (compared with placebo) of early Parkinson's. Like the GLP-1 drugs and α-synuclein trials, there was evidence for slowing the progression of motor symptoms. Indeed, benefit of a healthy person's fecal transplant highlights the role of the gut microbiome for this disorder. Beyond these three interventions with some success in small trials, there has been a tenfold acceleration of discovery of potent α-synuclein small molecule (pill) blockers with the help from AI.

Other approaches that are being assessed include cell therapy with induced pluripotent stem cells that can be tailored to whatever subtype of the disease the patient happens to have. Early clinical trials are giving us cause for optimism. Like Alzheimer's disease, there are ongoing vaccine clinical trials, directed against α-synuclein. Their success will ride, at least in part, on the ability to determine high-risk individuals.

As reviewed with Alzheimer's disease, a far better path than treatment once Parkinson's disease has taken hold would be prevention. The parallel strategy I've laid out for Alzheimer's (fig. 7.5) with partitioning high risk, close monitoring, and starting interventions early should be possible to assess in the near future.

## KEEPING THE BRAIN YOUNG

There has been considerable attention to new ways, beyond lifestyle+ factors, to preserve our brain health, and even achieve rejuvenation to a level of function

well below our chronological age. That interest is justified given multiple new studies that suggest this may someday be attainable.

Normal brain aging, without neurodegenerative disease, has many hallmarks that include neuronal dysfunction, especially in the hippocampus (where memory is choreographed); neuroinflammation (with microglia and astrocyte activation); blood-brain barrier leakage and vascular changes; and a precipitous decline in ability to regenerate nerve cells. We've also learned that chronic inflammation, such as occurs with degenerative knee osteoarthritis, accelerates brain aging. Intriguing are the rare brain "super agers" who at age eighty have the memory of people aged twenty or twenty years younger. By imaging, these individuals have less atrophy of brain white matter than their same-aged peers but little difference of lifestyle+ factors.

In mice, many of these hallmarks of aging can be replicated by giving old blood or plasma or transplanting aged blood (hematopoietic) stem cells to young mice. That is also the case with parabiosis, surgically connecting the blood circulation of old and young mice. There are many potential blood and cellular factors that may account for accelerating brain aging in young mice. When the opposite experiments are done, young to old mice, whether it be by parabiosis, plasma, blood, or bone marrow transplantation, there are improvements in neuronal, regenerative (new neurons, neurogenesis), and cognitive function, with less inflammation and more vascular density. These same benefits are simulated by exercise and caloric restriction. The pro-youthful blood factors that have been linked to improving brain-aging function include Klotho, platelet factor 4, and gonadotrophin releasing hormone (GnRH).

When platelet factor 4 was infused to old mice, there was notable reduction of neuroinflammation, improved neurogenesis, better strength of connection between neurons (synaptic plasticity), and less blood-brain barrier breakdown. Along with all these favorable changes, there was substantial improvement in cognitive function. Similar effects have been seen with pulsatile administration of GnRH, both in mouse models and in men with cognitive impairment from Down syndrome.

Klotho is a hormone that circulates in the blood, increases with exercise, decreases with age, is linked to longevity, and, due to its size, is unable to cross the

blood-brain barrier. One copy of the KLOTHO-VS gene (a variant of KLOTHO found in 20% of people) is associated with higher levels of klotho (the protein), longer life, improved brain volume, and cognitive function. In contrast, two copies decrease klotho levels. One copy has been associated with lower risk of Alzheimer's in people with the APOE4 allele.

When klotho is given to mice, there is an improvement of synaptic plasticity, memory, and cognitive function. A single intravenous administration of a low dose of klotho given to rhesus monkeys enhanced their spatial delayed response, a key metric for memory and cognitive function, not seen at higher doses, and persisted for two weeks. Unsolved from these experiments was the mechanism by which klotho could achieve this improvement, since it cannot access the brain.

Platelet factor 4 (PF-4) levels increase when klotho is given to mice, along with other platelet factors, as occurs with exercise. PF-4 readily crosses the blood-brain barrier. Intravenous infusion of either klotho or PF-4 to both young and old mice improved cognition. In mice whose PF-4 gene was knocked out, there was still an effect of klotho infusion, telling us that PF-4 is not the only factor that can potentially work as a messenger for brain health.

There's much more to the PF-4 story. PF-4 levels are higher in young mice and young people compared to their older counterparts. The PF-4 effect in mice, mediated through a cell receptor (CXCR3), reduced age-related hippocampal neuroinflammation, enhanced synaptic plasticity, and improved cognitive performance. The enhanced cognition with exercise in mice appeared to be mediated by platelets and replicated by intravenous infusion of PF-4, also demonstrating hippocampal neurogenesis in old mice. Use of PF-4 knockout mice showed this platelet factor was necessary and sufficient to achieve the beneficial brain effects. We're making major headway for identifying specific factors from the young in experimental models, which may ultimately lead to clinical trials in older people to preserve cognitive function.

GLP-1 drugs work predominantly through the brain and reduce brain inflammation. There are ongoing clinical trials testing these drugs to prevent progression in early Alzheimer's disease and Parkinson's disease, well beyond their current role in treating diabetes and obesity. It would not be at all surprising if such agents that safely and durably block brain inflammation, when given early

enough in people at high risk of neurodegenerative diseases, would ultimately render some protection. Our ability for identifying who and when is rapidly improving with the growing acceptance of biomarkers and use of multimodal AI to process all the layers of a person's data.

There are other novel approaches to promoting brain health in people. In five participants with mild Alzheimer's disease, the first senolytic clinical trial targeted brain cellular senescence using the combination of dasatinib, a tyrosine kinase inhibitor used in treating cancer, and quercetin, a natural flavonoid. This combination has been studied in experimental models and shown to be synergistic (beyond additive, as in 1 + 1 = 3) for the goal of inducing the death of senescent cells. After twelve weeks of therapy, blood and cerebrospinal biomarkers suggested a reduction in senescent cells. This first small foray trial without controls can be viewed as a *very* early step for possible senolytic therapy for preserving brain health.

Beyond these pharmacologic approaches, noninvasive device neuromodulation, with transient alternating current stimulation, has been shown to improve memory in people aged sixty-five to eighty-eight years. In a small randomized trial with sham controls, four days of treatment led to sustained benefit in memory one month later.

As these approaches go forward in clinical trials with controls, we will be able to determine brain-specific changes of aging using plasma proteins and MRI imaging. That'll facilitate a determination of whether they are working, promoting brain health, slowing the progression of aging, or even achieving a rejuvenating impact.

Before moving on, just a quick note about nootropic supplements that are purported to improve learning and memory, such as Alpha Brain, Neuriva, Kin Euphorics, several amino acids, herbal extracts, probiotics, and other substances. None of these have any meaningful clinical trial data to support their use.

Countering the neurodegeneration that occurs with aging, no less with Alzheimer's and Parkinson's, remains one of the most formidable challenges for promoting health span. I think of it as one of the big killers in the second half of our lives because extending lifespan without preserving cognitive function is not appealing to anyone. Fortunately, from the culmination of a large body of recent

studies, we may be on the cusp of breaking through with new ways to promote brain health so that when we reach advanced age, we're cognitively crisp.

The implications of the current historic convergence of breakthroughs in medical science are broader than is easy to grasp for even the sharpest mind.

# PART III

Great Implications

# 8

# Curing Rare Diseases

You might ask why a book on health span would include a chapter about rare diseases. It's because our current approaches to curing rare diseases will play an increasing role in managing common diseases. The most consequential life science breakthrough of our era is genome editing, which is already being applied for patients with cancer and heart disease. That's just the beginning. At the very least, your children or grandchildren may undergo some form of genome editing during their lifetime. But before we can fast-forward to ponder the future, let's get grounded. Here and now, the big picture is that "rare" diseases, cumulatively, are common.

Six percent of the world's population suffers from about ten thousand rare diseases, which equates to well over four hundred million people. The vast majority of the diseases, more than 80 percent, have a genetic basis. According to the European Union, a rare disease is one that affects less than one in two thousand individuals, and an ultra-rare disease has a prevalence of less than one in fifty thousand people. The US FDA categorizes a rare disease as occurring in less than one in two hundred thousand people. The term *hyper-rare* has been applied to prevalence of less than one in one hundred million. So, there's clearly a wide range of terms and definitions that span several orders of magnitude. But even conservative estimates of the cumulative proportion of those affected by diseases

most people have never heard of is between 3.5 and 5.9 percent. More than one in twenty? That's a lot.

Let's run through an example of how a new approach for a rare genetic disorder may ultimately affect an extremely common disease. Homozygous familial hypercholesterolemia, with a prevalence estimated at no more than one in three hundred thousand, results from two copies of genes with mutations that markedly elevate LDL cholesterol levels (three genes—PCSK9, the LDL receptor, and ApoB—account for most cases). This genetic disorder leads to LDL cholesterol levels over 400 mg/dl, at least fourfold the normal range. Without early diagnosis and aggressive therapy, premature coronary artery disease and death can occur. People with one copy of the gene are heterozygotes or carriers, comprising 1 in 250 people. That's relatively common, well below the threshold of 1 in 2,000 for the European Union definition of a rare disease. We currently have an array of treatments to get the LDL cholesterol down, which, without treatment, is usually >190 mg/dl in adults or >160 mg/dl in children. Those lifelong treatments are statins, ezetimibe, PCSK9 antibodies or inhibitors, and bempedoic acid. Sometimes they are not tolerated well, with significant side effects, or do not adequately reduce the LDL cholesterol level. What if there was a one-shot CRISPR gene editing treatment that would markedly lower the LDL levels for life?

After a single injection by vein of a mRNA nanoparticle package, containing a CRISPR base editor that knocks out the PCSK9 gene, there was substantial and durable reduction of LDL cholesterol in monkeys. That led to the first clinical testing in New Zealand, and soon thereafter the FDA gave a green light for the company Verve Therapeutics to proceed in people with heterozygous (not homozygous) familial hypercholesterolemia, which has, in clinical trials, provided very encouraging results. While it's still early to know for sure, we can envision—if it works and is safe—that people with a high risk of coronary artery disease and heart attacks, a disease that kills about seven hundred thousand Americans each year and is the number one killer worldwide, would be potential candidates to get genome editing. That illustrates how a new approach to an ultra-rare genetic disorder could be ultra-relevant.

CRISPR technology is rather famous these days, but what is it exactly, and where did it come from? In the 1980s, sequencing the genes of bacteria led to the discovery of unusual DNA stretches that were called CRISPR and were later found to be part of bacteria's defense system against viruses. The unusual palindromic repeat stretches of bacteria DNA were coded for RNA that matched invading virus genes and helped destroy them. This natural defense system has been around for billions of years. But in 2012, a paper in *Science* with the arcane title "A Programmable Dual RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity" woke us up to a new possibility in life science: CRISPR could precisely edit DNA in test-tube experiments using a guide RNA and the Cas9 molecular scissors enzyme. (Cas stands for CRISPR-associated proteins.) Like the seminal 2005 discovery by Katalin Karikó and Drew Weissman for blocking mRNA-induced in vivo inflammation, it attracted little notice initially, but later it was the basis for a Nobel Prize awarded to Jennifer Doudna and Emmanuelle Charpentier. Within a year of the CRISPR discovery, there were multiple reports of precise DNA editing in animal and human cells.

Before CRISPR, we had tools that cleaved DNA nucleotides known as nucleases to do genome editing, including zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), but they were difficult to program to achieve precise editing.

Over the next decade, CRISPR technology underwent intensive refinement to improve the accuracy and precision of editing, reducing the chance for off-target effects. All three of these nucleases (ZFNs, TALENs, CRISPR-Cas 9) cut the double-stranded DNA into two pieces. The cell wants to repair the broken ends of chromosomes, which it can do accurately about 90 percent of the time. That leaves a small percentage of mistakes in the rejoining repair process. But the nuclease keeps cutting, and the repair process keeps following, accumulating more mistakes. The cycle continues until the nuclease no longer recognizes the altered, mistake-laden sequence, with nucleotide insertions and deletions, ultimately achieving disruption of the gene. The downside from this double-strand break and repair process is the lack of control of the resultant sequence with random insertion and deletions (fig. 8.1). It's a disruptive gene-knockout strategy that can't be used to fix a gene. Let's call this CRISPR 1.0.

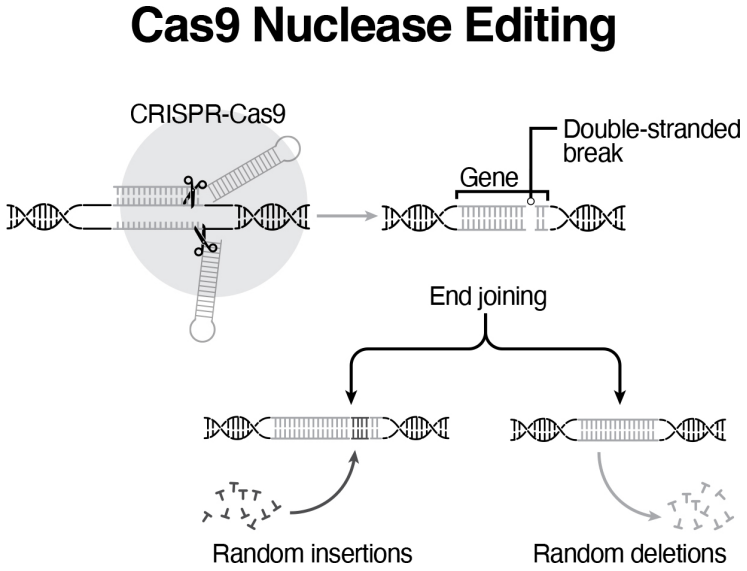



***Figure 8.1.*** CRISPR 1.0 achieved by double-stranded DNA breaks with the innate repair process that can lead to random insertion or deletions. Adapted from Joy Wang et al., "CRISPR technology: A decade of genome editing is only the beginning," *Science* 379, no. 6629 (January 2023): eadd8643, https://doi.org/10.1126/science.add8643. Description 41

But with refinements, the fixing can be far more precisely focused. With base editing (fig.8.2), instead of a double-strand DNA break, a single strand is nicked, enabling a single base pair change like an A to a G and C to an A. An even more versatile single-strand editing incorporates an enzyme called reverse transcriptase and a prime editing guide RNA that directly copies edited information to the target DNA site, as used by Verve Therapeutics. David Liu of the Broad Institute has likened it to a word processor: "It allowed researchers to turn any letter into any other letter and delete or insert stretches dozens of base pairs long. In theory, it could debug around 90 percent of all known disease-causing mutations." These DNA editors with enhanced nondisruptive, fixing capabilities have been called CRISPR 2.0. They include other emerging editing tools for large deletions (CRISPR-Cas 3) and insertions (CRISPR-associated transposases), along with RNA and epigenetic editors (fig. 8.2). Another discovery from nature was "bridge RNA" molecules, a group of programmable recombinases, enabling precise editing of long DNA sequences.

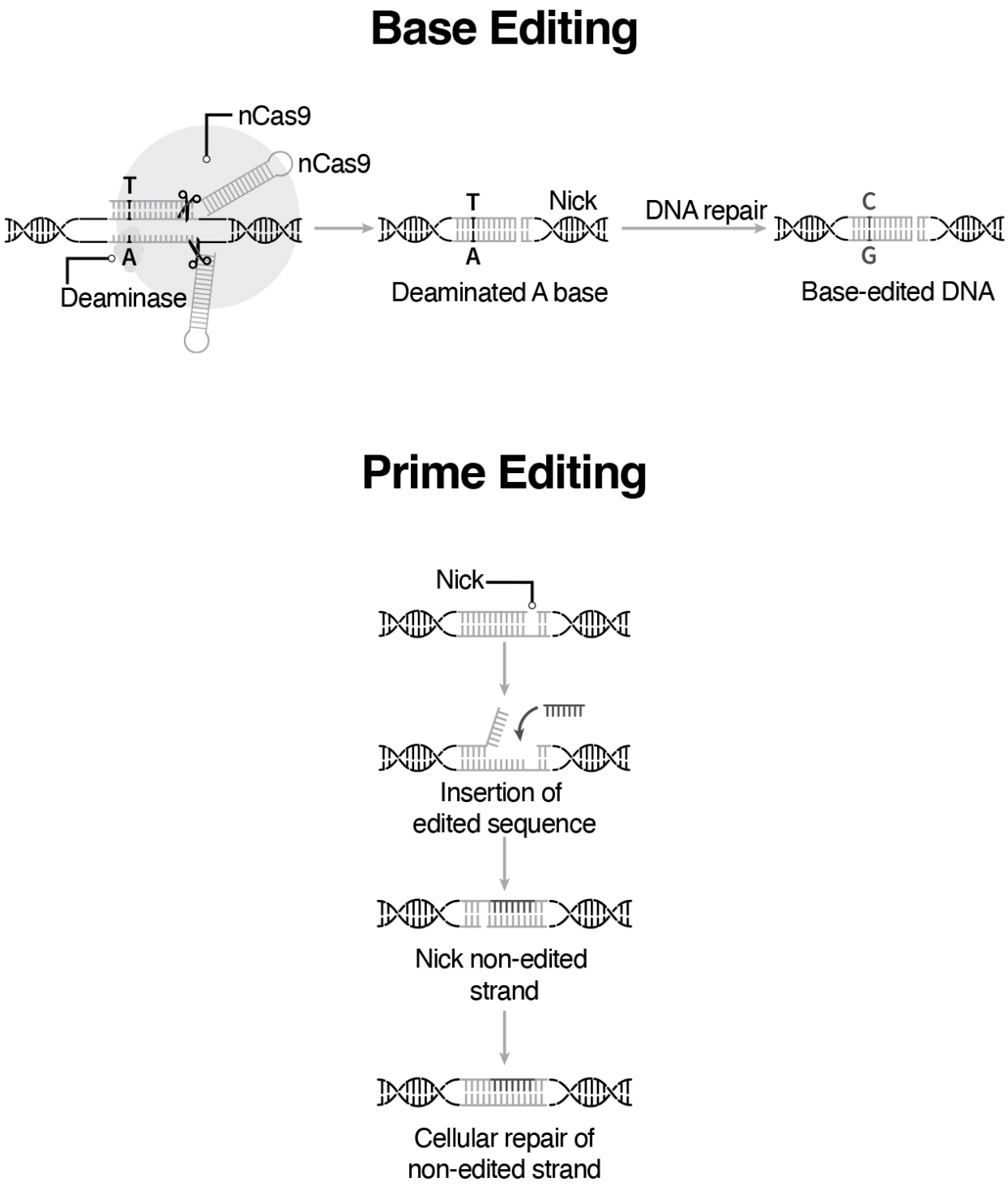



***Figure 8.2.*** CRISPR 2.0. Base editing (upper) with single-strand DNA nick and substitution of intended base pair. Prime editing (lower) with single-stranded DNA nick and insertion of a DNA sequence. Adapted from Joy Wang et al., "CRISPR technology: A decade of genome editing is only the beginning," *Science* 379, no. 6629 (January 2023): eadd8643, https://doi.org/10.1126/science.add8643. Description 42

In late 2023, just over a decade after CRISPR was discovered, a major milestone was achieved with the regulatory approval by the United Kingdom and United States for genome editing to treat sickle cell anemia, called Casgevy, for relief of debilitating sickle cell crises that affect some one hundred thousand Americans, mostly African Americans. The United Kingdom also approved CRISPR editing for beta-thalassemia, markedly reducing the need for blood transfusions. For both diseases, there had been substantial warm-up using CRISPR 1.0 to demonstrate a disruptive workaround strategy that does not fix the genetic defect in the hemoglobin gene (a simple T for an A switch mutation) but instead targets a gene called BCL11A to restore production of fetal hemoglobin. The marked and durable rise in fetal hemoglobin following CRISPR is what can provide a definitive treatment for both sickle cell anemia and beta-thalassemia.

But it's an extremely complicated treatment that requires the patient to first undergo a collection process of blood-producing stem cells, using an apheresis machine that separates out blood cells into components, and takes several hours, often requiring transfusions subsequently. Then chemotherapy is given to wipe out the bone marrow (which can lead to infertility) for eventual replenishment with the patient's edited stem cells. That precipitously drops the patient's white blood cells, requiring hospitalization under sterile conditions for weeks to months. Eventually, the patient's blood stem cells exposed fully to CRISPR-Cas9 (via electroporation) are infused, but the patient must stay in the hospital until the immune system recovers.

Although this was a huge step forward for genome editing to provide medical treatment, there are two fundamental limitations of this first approved strategy. It's a disruptive knockout of a gene, not fixing the underlying genetic defect, because it relies on CRISPR-Cas 9, or CRISPR 1.0. Also, the patient must go through a long, grueling process because the editing is done in cells outside the body (ex vivo). The future of genome editing will move to in vivo, in the patient's body, ideally with a one-and-done shot like I touched on for familial hypercholesterolemia that uses base editing, not Cas-9. Indeed, a clinical trial of base editing of sickle cell disease to fix the single base pair mutation is underway. Even pills are being developed to avoid the need for shots. One pill that

epigenetically targets the same BCL11A gene has been shown to be effective in mice and monkey models of sickle cell disease.

When CRISPR got FDA approval for sickle cell disease, a gene therapy called Lyfgenia was approved for the same disease. While the terms *gene editing* and *gene therapy* are often used interchangeably, they are two very different strategies.

When I asked my friend Fyodor Urnov, a pioneer in gene editing, to explain the difference to me, he said, "Imagine you have a car with a flat tire. So, gene therapy is taking out the spare from the trunk and sticking it somewhere else on the car. So now the car has a fifth wheel and hoping it runs. And believe it or not, that actually works. Gene editing is fixing the flat."

The metaphor speaks well to the fact that gene therapy is a gene addition, unlike genome editing, which disrupts or fixes a specific gene. It does not correct the underlying defect and has traditionally relied on an engineered virus delivery system, such as the adenovirus or lentivirus vectors.

About fifteen different gene therapies were approved in the years before CRISPR's Casgevy, beginning in 2012 with the European Medicines Agency approval of Glybera for lipoprotein lipase deficiency, and by the FDA in 2017 with Kymriah and Yescarta to genetically engineer T cells in people with lymphoma, and Luxturna for a rare genetic eye disease, Leber's congenital amaurosis. The approvals have greatly expanded since to spinal muscular atrophy, hemophilia A and B, beta-thalassemia, Duchenne muscular dystrophy, severe combined immunodeficiency ("bubble boy disease"), metachromatic leukodystrophy, dystrophic epidermolysis bullosa, and several others. Single-injection gene therapy to restore hearing in children with autosomal recessive deafness 9 has been demonstrated without adverse side effects, which could be the start of using gene therapy to prevent or treat a wide array of hearing loss conditions including presbycusis (hearing loss with advanced age). Ongoing clinical trials for central nervous system conditions are targeting amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease.

This field is more than a decade ahead of genome editing for getting treatments out there. But the intrinsic limitations of gene therapy include its use restricted to recessive mutations, inability to deliver large genes, and the unregulated nature of the additional gene copy that is introduced (without native

promoters and enhancers that regulate gene expression). The virus vector for the gene therapy delivery has the potential to integrate with DNA of the recipient as well as inducing inflammation. Nonetheless, many gene therapies have fulfilled critical unmet needs for people with rare diseases and have paved the way for a more precise and potentially curative approach.

## ONE-SHOT GENOME EDITING

There has been a long string of success in experimental models, such as a single injection of base editors, delivered in vivo (injected into the animal) with an adeno-associated virus (AAV) vector for progeria, a devastating disease of accelerated aging, replacing a C-G base pair with a T-A. Likewise, in vivo base editors were used to restore hearing in a mouse model of a dominant hearing loss. CRISPR-Cas 9 injected to the brain with AAV in a model of familial Alzheimer's disease reduced amyloid deposition and microglial activation, and improved cognitive performance. Prime editing of phenylketonuria, a metabolic disease of the liver, was also successful given in vivo, but there was requirement for high vector doses that induced immune response. Direct injection of base editors with AAV into the chest to treat hypertrophic cardiomyopathy, a genetic disorder that occurs in one of five hundred people, achieved 30 to 60 percent editing efficiency (proportion of heart cells fixed) and improvement in heart function and structure. Use of base editors, directly injected to the heart, has been shown to protect from lack of blood supply, which could be useful in people with prior heart disease and heart attacks.

While the above were all using mouse models, base editing moved on to primates to test correction of the PCSK9 mutation that is a frequent culprit for familial hypercholesterolemia in people. LDL cholesterol was reduced by 65 percent after a single shot of base editors was delivered in a lipid nanoparticle package into a vein, and this effect was durable for as long as the monkeys were assessed (beyond one year). That strategy has gone on to clinical trials, with results similar to the nonhuman primates.

That's a good transition to the other clinical trials for genome editing, all of which were antedated by animal model work. They include CRISPR-Cas9 for

treating hereditary amyloidosis, a life-threatening condition due to a misfolded protein called transthyretin (TTR) that accumulates in the heart and nervous system. The disruptive editing approach used a lipid nanoparticle delivery, the first genome editing given to people by vein, targeting the liver where the protein is made, and achieving marked (more than 90%), dose-dependent, and durable reduction of TTR in the blood.

Another life-threatening condition is hereditary angioedema, characterized by unpredictable and severe swelling attacks. Here again, a single infusion of the CRISPR-Cas 9 nanoparticle package targeting a kallikrein gene (KLKB1) achieved a potent, dose-dependent, and durable lowering of kallikrein levels and less angioedema attacks. Trials with base editing and Cas12a are showing good results for sickle cell disease, with plans to move to direct, in vivo injection rather than the cumbersome ex vivo approach currently approved.

That's not the only use of genome editing to deal with the immune system. By editing multiple genes that would lead to transplant rejection, pig organ transplantation of hearts and kidneys to patients have become possible and will potentially, if proven safe and providing durable benefit, greatly alleviate the shortage of donor human organs. Clinical trials in participants with human immunodeficiency virus (HIV-1) were initiated by Excision BioTherapeutics, with CRISPR-Cas9 to cut at two sites within the HIV genome to eliminate HIV from cells, but initial results were disappointing. For type 1 autoimmune diabetes, CRISPR is being used to edit immune-related genes to prevent the pancreatic islet cell transplant, made from the individual's stem cells, from being attacked.

Success with in vivo correction of cystic fibrosis in the experimental model has helped propel the start of clinical trials of genome editing with an mRNA and nanoparticle package for this condition. If a one-and-done editing approach works, it might simulate the very high efficacy of Trikafta therapy that requires lifetime daily dosing.

Targeting cells outside the body (ex vivo) for editing is not as difficult, which is why the first approvals have come for sickle cell disease and beta-thalassemia. And the many uses for cancer are exemplified by clinical trials of base editing a healthy person's T cells at multiple sites and using this as an off-the-shelf treatment for T

cell leukemia, a rare cancer that is difficult to treat. Engineering T cells to drive a more potent immune response against solid cancers has moved forward in clinical trials with multiple neoantigens.

A recent outgrowth of rare disease omics is the finding that in people with Laron syndrome, a cause of dwarfism in fewer than five hundred people in the world who have a considerably lower risk of heart disease, diabetes, cancer, and other age-related diseases, their insulin-like growth factor IGF-1 levels are quite low. That substantiated the IGF-1 gene's role in the aging process and increased the interest for a targeted drug intervention.

All of this looks encouraging but there are many hitches. It has been shown that base and prime editors can still induce double-strand DNA breaks, with potential genotoxicity of the unintended by-products, albeit at a much lower frequency. There is significant concern about off-target effects for all types of genome editors: cleavage or nicks in DNA that are unintended because the guide RNA doesn't have perfect specificity. Fortunately, they occur less frequently with base or prime editing. But permanent edits in a person's genome in some cells, even though they are typically small insertions or deletions, can have large consequences. When I spoke to David Liu, the inventor of base and prime editing, he nicely contextualized this issue:

> Virtually every substance we've ever put into a person, including just about every medicine we've ever put into a person, has off-target effects, meaning [the substance] modulates the function of biological molecules other than the intended target. Of course, the stakes are higher when those are gene editing agents because those modifications can be permanent. I think most off-target edits are very likely to have no consequence because most of our genome, if you mutate in the kinds of small ways—like making an individual base pair change for a base editor—are likely to have no consequence. We sort of already know this because we can measure the mutational burden that we all face as a function of living and it's measurable, it's low, but measurable. I've read some papers that estimate that there are roughly thirty-seven trillion cells in an adult person, that there are billions and possibly hundreds of billions of mutations that accumulate every day in those thirty-seven trillion cells. So, our genomes are not quite the static vaults that we'd like to think that they are. And of course, we have already purposefully given life extending medicines to patients that work primarily by randomly mutating their genomes. These are chemotherapeutic agents that we give to cancer patients.

In engineering T cells with CRISPR-Cas9 for cancer therapy, extra or missing chromosomes appeared early almost 10 percent of the time. Could we be

promoting cancer? When CRISPR-Cas9 was coupled with an adeno-associated virus vector, insertions of pieces of the virus genome were found in the T cells. Yet another worry is unintended editing that takes place in a person's ovaries or testes, as has been noted in nonhuman primates, which could potentially lead to germline transmission of renegade DNA in eggs or sperm. Meaning, we would pass along the negative consequences to our kids. And their kids.

It's not just the cut and nick DNA edits that are the worry. There are potential reactions to what the editor and guide RNA are packaged in. An immune reaction to an adenovirus-associated virus vector used in CRISPR genome editing of a patient with Duchenne muscular dystrophy was implicated as the cause of death, the first patient to die after genome editing.

For ex vivo editing used for the approved treatments of sickle cell disease and beta-thalassemia, the patient must undergo busulfan therapy that wipes out the bone marrow in order to make room for the edited blood stem cells. That chemotherapy comes with downstream risk of cancer. It is unsurprising that the FDA has warned on the possible risks of cancer with gene therapy for engineered T cells, and this caution should extend to all forms of genome editing too. Long-term surveillance is needed alongside mitigating these risks, with better editors and delivery methods. While prime editing is currently considered the most versatile form, its efficiency has been shown to be markedly enhanced.

Ideally, the field will move to the in vivo, one-shot, in the body route, since ex vivo is laborious, expensive, and typically necessitates a considerable time delay to intervention. It seems riskier in principle. For now, however, genome editing in the body is greatly constrained by its reach, to the blood, which also accesses the liver, or by direct injection into the eye or ear. But there's a blueprint for improved delivery that will ultimately help us reach the goal of broad access throughout the body.

In vivo access to most parts of the body will rely on better nanoparticles and virus-like particles. But even with what we have now, there has been successful restoration of a critical missing enzyme to patients with a rare disease using an mRNA that codes for it packaged within a nanoparticle, with no editors. However, unlike what can be accomplished with editing, it requires repetitive injections.

The importance of nanoparticles can't be emphasized enough, aptly put by Megan Molteni, for a liver gene target:

> Precious CRISPR instructions were billions of microscopic balls of fat, each enrobing an mRNA's rigid backbone like a sticky, squishy sock. These lipid nanoparticles, or LNPs, work like sebaceous sherpas, ferrying their genetic cargo past peril after peril. To get where it needed to go, the CRISPR-encoded mRNA would have to withstand being buffeted about in the bloodstream where immune cells roved, then fan out into the forking network of vessels winding through the liver, slip through a pore in those vessels' walls, fuse with the surface of a hepatocyte, get sucked across its protective membrane into an endosome, and somehow escape before the endosome became mRNA-meltingly acidic. Only then could CRISPR even have a chance to do its job.

Every one of the four key components of nanoparticles (fig. 8.3) is being worked on for improved stability and reach. We've already seen the enhanced potential for gold nanoparticles to get editing done in blood stem cells. Synthetic nanoparticles, such as adding a fifth lipid or an attached (conjugate) antibody, have now been enabled, getting editors into the brain, spleen, and muscle with ongoing work to get them to the heart, lungs, and bone marrow.

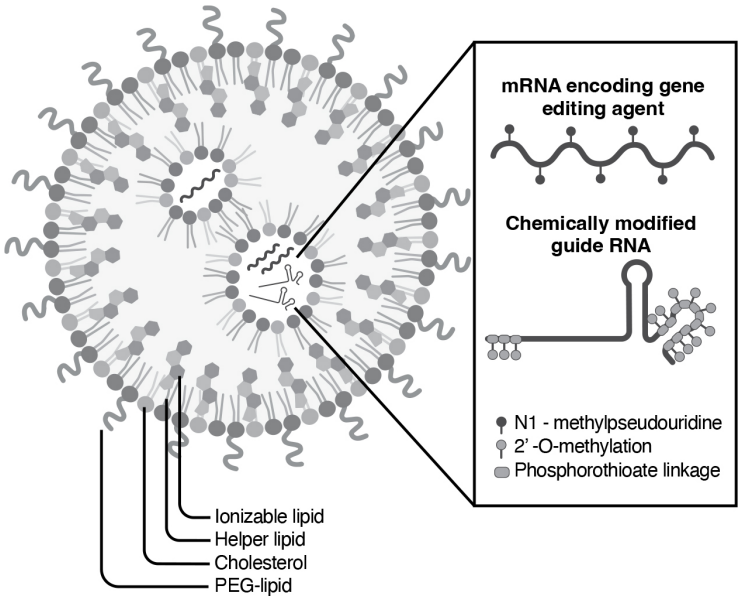



***Figure 8.3.*** The anatomy of nanoparticles, with multiple layers of lipid, packaging the mRNA. Adapted from Aditya Raguram et al., "Therapeutic in vivo delivery of gene editing agents," *Cell* 185, no. 15 (July 2022): 2806–27, https://doi.org/10.1016/j.cell.2022.03.045. Description 43

The other main direction is engineering the delivery vehicle, the broader term of enveloped delivery vehicles (EDVs), or more specifically, virus-like particles

(VLP), to hold much more cargo and enhance its release at the target. For virus-like particles, the virus is gutted out, making it noninfectious and providing increased room for mRNA or proteins. There's been progress on multiple fronts, such as in vivo liver delivery with base editors or retina delivery of prime editors with 65- to 170-fold increase efficiency. An advantage of VLPs with high efficiency is their short exposure time, which reduces the potential for off-target editing. Besides using human proteins as a capsule structure around the editor and guide, there's even been use of bacteriophage to make better artificial viral vectors with enhanced cargo capacity. With all these tactics for improving efficiency and reach of delivery throughout the body, we'll surely see progress in the future. What's next?

As Fyodor Urnov, a pioneer in the field, who coined the term *genome editing*, and professor at the UC Berkeley Innovation Genomics Institute, told me, the lung, muscle, and spine will be the next three organ systems deemed approachable. CRISPR-ing the spine is especially noteworthy as a treatment for chronic pain by blocking the spinal neurons from transmitting the pain signal. The new editing frontiers won't stop there. Genome editing in utero has started. Eventually it will be possible for the gene-edited brain to self-edit unfavorable alleles like APOE4, but that's still years away. And one day all this will be done by a pill rather than an injection. That's just a matter of time and building on drug delivery innovations.

## BEYOND DNA EDITING

Most of what has been discussed to this point pertains to DNA editing, but the same principles apply to RNA, microbiome, epigenetic, and mitochondrial editing. RNA editing has several advantages because it doesn't change proteins, the effect is short-lived, and the transience, lack of permanent impact, may offset concerns about off-target effects. RNA editing has been approved by the FDA for treating Stargardt disease, a rare eye disease that causes macular degeneration and loss of vison. It is being pursued to treat alpha-1antitrypsin deficiency and as a treatment of liver (hepatocellular) cancer. Here, again, what was envisioned for

rare disease treatment may have much broader applicability to alter the RNA sequence in cancer that leads to cell death of tumor cells.

Considering the results in animal models, editing the epigenome (fig. 8.4) is now drawing considerable attention. Like RNA editing, it does not change a protein directly; rather, it controls the regulation of a gene, the on-off switch, and it's temporary. It's a hit-and-run approach. The term *gene tuning* has been applied because, like adjusting dial settings on a soundboard, epigenome marks can be modified to harmonize the expression of multiple genes at once, not just turn them on or off. The putative enhanced safety profile is alluring, as are the results of mouse studies for cholesterol lowering, Alzheimer's, Huntington's disease, Dravet syndrome (a rare inherited form of obesity), modulating a pain perception gene, and permeating the brains of mice to silence neuron-killing prions. But many of these studies used AAV as the ferry, which undermines the objective of a transient effect. Other cargo techniques are being explored. Intranasal delivery of exosomes, loaded with an epigenetic editor, was successful for reducing expression of beta-amyloid production in brains of mice. Epigenetic silencing of the PCSK9 gene in mice led to marked and durable cholesterol lowering with nanoparticles, which augurs well for avoidance of AAV delivery. With a full atlas of twenty-eight million methylation changes across cell types, a new company (Moonwalk Therapeutics) plans to edit methylation sites to treat chronic disease and modulate the aging process. Other companies formed to edit the epigenome are focused on treating liver cells in patients with hepatitis B or cancer. Clearly, these are ambitious programs that, if successful, would have transformative impact well beyond rare genetic disorders.

Which brings us to a familiar therapeutic channel. Engineering the gut microbiome has enormous potential for modulating our immune response and inflammation throughout the body. There are many ways to manipulate the gut microbiome, from the most primitive of fecal transplant, to perhaps the most advanced using gene editors, that design or change the function of bacteria and other microbial residents in our gut. One example of several companies is a bacterial strain to break down oxalate in the gut, to reduce the development of kidney stones (most commonly caused by calcium oxalate). Theoretically, it would be possible to get gut microbes to secrete anti-inflammatory compounds,

or block ones that promote inflammation, or tackle antibiotic resistance. The first successful editing of gut bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) was accomplished with base editors and an engineered bacteriophage (a virus that infects bacteria) that achieved over 90 percent editing efficiency after a single dose in mice. The first microbiome editing project undertaken by Jennifer Doudna and UC Berkeley's Innovative Genomics Institute is childhood asthma, for which a culprit bacteria is known, but an approach to specifically edit it with a pill or oral administration is not. It will be challenging but may be possible. In the meantime, editing the gut microbiome of cows, targeting archaea single-cell organisms that produce the methane, will likely be an earlier application that could, if highly effective and durable, dramatically reduce greenhouse gas emissions.

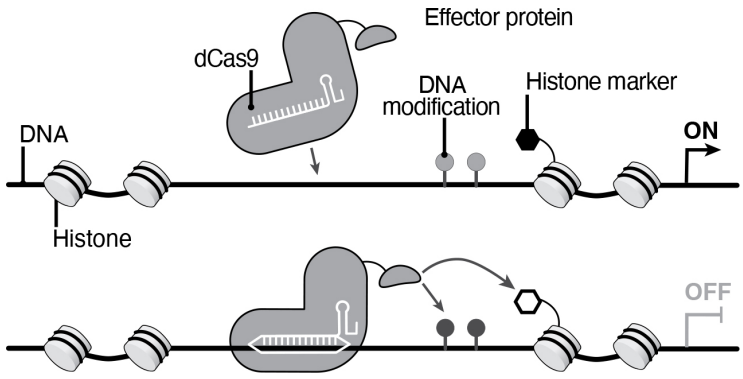



***Figure 8.4.*** Epigenetic editing. Modulating the expression of a gene (on or off) by an "effector" protein that adds or removes chemical tags on DNA and histone proteins. Adapted from Jocelyn Kaiser, "Better than CRISPR? Another way to fix gene problems may be safer and more versatile," *Science News*, June 1, 2022, https://www.science.org/content/article/better-crispr-another-way-fix-gene-problems-may-be-safer-and-more-versatile. Description 44

We turn back to editing DNA, but now it's not in the cell nucleus; it's in the mitochondria (mtDNA). There are nearly one hundred disease-causing mtDNA mutations that are maternally inherited, affecting about one in five thousand individuals. Getting into the mitochondria is tricky because of their double membranes, but with adapted nucleases and editors, increasing success is being reported in lab studies. In theory, mitochondrial editing has potential relevance in medicine for the pivotal role this organelle plays in the aging process. For now, it is contemplated to be the preferred approach to mitochondrial genetic disorders

compared with replacement therapy that requires a donor maternal egg to substitute the mitochondrial genome for the biologic mother's (thus delivering a "three-parent baby"). This takes us to germline (egg, sperm, embryo) instead of somatic (body cell) editing, which, as we know, has a special peril.

## ETHICAL, LEGAL, AND EQUITY QUESTIONS

In 2018, He Jiankui, a rogue Chinese scientist, announced, initially via YouTube videos, that twin babies had been born after he had CRISPR edited a human embryo and implanted it in the mother's uterus. This was the first human germline editing—not just inducing a mosaic of the edits across the trillions of cells of a person's body but enabling transmission to the next generation. It shook the science community. Days after the announcement, in a *New York Times* invited op-ed, I called it "a misguided, reckless use of powerful gene-altering tools to create edited human beings. We should not proceed down this road until we know far more about the consequences of what we are doing."

Later, we learned Jiankui had actually edited thirty-one embryos, and six women were implanted. All of this was done without approval of the hospital in Guangzhou. Jiankui was imprisoned for three years for his unethical conduct and illegal medical practices and fined nearly half a million dollars.

Someday there will be a place for heritable human genome editing, but it's still a ways off. While double-strand breaks are obligatory, worrisome large deletions, loss of chromosome segments or arms, mosaicism, and off-target edits have all been seen in lab studies of CRISPR-edited embryos. Until these potentially serious genetic defects—that will be transmissible—can be satisfactorily addressed, the trade-off for treating (and potentially curing) rare monogenic diseases isn't acceptable.

The cost for Casgevy, the FDA-approved CRISPR genome editing for sickle cell disease, is $2.2 million; the gene therapy approach for the same disease has a list price of $3.1 million. The estimate to treat a patient with sickle cell disease using current therapies for a lifetime is about $1.7 million. But that estimate doesn't convince payors to pick up the huge up-front cost, which for some gene therapies exceeds $4 million, the most expensive treatment in the world. There

are many ideas for how the costs could be reduced or justified, and there's an ethical obligation to provide these treatments, especially for those least likely to gain access. While much has been written, there's no solution in sight for this chasm between a momentous medical breakthrough and profound inaccessibility. One major step is to reduce the multiple years it takes for FDA review to "create a more streamlined path for bringing much-needed CRISPR medicine tailored to patients with a one-of-a-kind genetic typo." Collaborations between academia with industry to radically reduce the cost of manufacturing are starting to take hold. It will take time and a multipronged aggressive strategy, as the Innovative Genomics Institute published. Hopefully, in the years ahead, the barriers to getting genome editing for all who would benefit will be overcome.

Reading human genomes has become tremendously cheaper since we first learned how to do it in 2003, when it cost nearly $3 billion. We call it sequencing. The cost of a whole genome sequence is in the range of $200 in 2025, and it can be done in as little as three hours. This technology is grossly underutilized in clinical medicine. For sick newborns and babies who are critically ill without a diagnosis, a rapid-sequence identification of disease-causing mutation and a clinical management program can all be accomplished in eleven hours or less, utilizing multiple AI software packages, and have saved the lives or vital organs of hundreds of babies. The AI tools provide an exceptionally rapid and accurate means of identifying which of the millions of DNA variants may be implicated in a child's condition and reviewing all the known medical literature and potentially useful treatments for the causative mutation.

A similar approach has been used in adults who are in the intensive care unit with an unknown diagnosis, using sequencing to crack the case within five to seven hours. My team at Scripps Research and those at several other academic centers have offered whole genome sequencing as a way to unravel the diagnosis when the patient has a serious and chronic unknown condition. We've applied that research program for hundreds of individuals and have had a success rate of about 40 to 50 percent for getting to the root molecular cause, often identifying a treatment that can help the patient's condition. Another important application of sequencing is known as "molecular autopsy." The concept is that a sudden unexpected death in a person younger than forty to fifty years old warrants whole

genome sequencing of the deceased for a molecular diagnosis. If a gene mutation is identified that has been implicated with sudden death, children can also undergo sequencing. This approach can be both lifesaving for descendants of the deceased and reassuring for offspring to know they do not harbor the genomic susceptibility.

The genomic "fast lane" for determining cancer diagnosis during surgery is an exciting initiative that is starting to get traction. For example, in Melbourne, Australia, in 2024, just forty minutes after a biopsy was obtained using a portable sequencing device, forty-five of fifty tumor samples were accurately classified for cancer type. Whether to proceed with aggressive surgery or not was clear. In the years ahead, whole genome sequencing will become more routine for assessing risk.

Genomics England is conducting a research project of sequencing two hundred thousand babies, not just to find rare genetic diseases but also to predict risks of adult conditions and define drug sensitivities. Similar programs of newborn sequencing have been launched in the United States (one hundred thousand newborns), Belgium (forty thousand newborns), Greece, Australia, France, and the European Union. Tens of thousands of healthy newborns are being sequenced for four hundred genetic diseases that have treatment options through a consortium coordinated at Rady Children's Institute for Genomic Medicine, a research partner of ours. The combined features of low-cost, rapid, and accurate data interpretation with AI as well as emerging evidence for improving patient outcomes all point to increasing use of reading genomes for medical diagnosis and care.

# 9

# Controlling Our Immune System

We've discussed ways that we can empower our immune response to meet all kinds of threats to our health span. Though not generally appreciated, the inverse of that—stopping our bodies from attacking themselves—is one of the most important simultaneous advances in life science. About eighty different autoimmune diseases affect more than 10 percent of the population. They include rheumatoid arthritis, Crohn's disease, and lupus; all are currently treated with lifelong, broad immunosuppressive drugs that usually do not adequately control the conditions. These treatments pose risk of serious infections and, moreover, loss of therapeutic efficacy due to the immune system building resistance to attempts to control it. Our immune system is, after all, evolving.

Just like the breakthrough for obesity with GLP-1 drugs that took many decades, early attempts to achieve control over our immune system began in the 1950s. They all basically failed. Only now are we starting to see remarkable results in conditions like systemic lupus erythematosus, type 1 diabetes, and rheumatoid arthritis. By reprogramming the immune response, multiple strategies are being pursued to make our immune system more tolerant of what appears threatening to it. (The word *tolerant* is key since these approaches are known as tolerogenic.) This could, and really should, lead to prevention or definitive treatment for these conditions—even a cure.

This bold goal is realistic due to dramatic improvements in our understanding of the complexity of our immune system. It's one of the most complex systems in our body, if not the most complex. For an in-depth and highly accessible review of its workings, I recommend Phillip Dettmer's book *Immune*. For now, here is a primer.

## BIOLOGY OF THE IMMUNE SYSTEM

In one drop of blood, we have about 400,000 immune cells and 13 trillion antibodies, admixed with approximately 250 million red blood cells and 15 million platelets. The magnitude of immune cells and antibodies conveys the priority that drop of blood and our whole body puts on the defense against all sorts of insults and attacks. The first line of defense is the innate system, able to rapidly attack and reliably distinguish our cells ("self") from foreign invaders ("other"). The innate system also gathers intel and determines whether the second line of defense, the system that adapts to unfamiliar foes, needs to be activated. The innate system's epithelial cells, such as our nasal mucosa, release interferons when exposed to a respiratory virus. This system also includes a variety of nonspecialized types of white blood cells, including macrophages, neutrophils, dendritic cells, monocytes, mast cells, basophils, eosinophils, and natural killer cells. Macrophages are present in every organ of the body. Natural killer (NK) cells, protecting us from harmful invader pathogens, were first described fifty years ago and are now being tapped as a strategy for cancer immunotherapy.

In contrast, the adaptive system is specialized beyond merely distinguishing between self and other. It takes longer to respond, with a line of B cells (B from bone marrow) and plasma cells that manufacture antibodies at whatever the needed scale, and many types of T cells (T from thymus) that are helpers (CD4+), regulators (Tregs), or cytotoxic cell-killers (CD8+). Both B and T cells have memory lineages that have an exquisite ability to recognize invaders they have seen before, even long, long before in the life of that individual. Untrained B cells and T cells are called "naive," because they are immature, haven't seen their target antigen, and aren't yet activated. But when these cells have been trained to

remember invaders, they are called memory B cells and T cells. While antibody and cell-mediated immunity are often discussed separately, they are inextricably linked. There's intense crosstalk between the many different types of cells and all parts of the innate and adaptive immune systems.

The total package and distribution of immune system cells has been quantified, as seen in figure 9.1. Dendritic cells, with their unique morphology of branched tentacles, are concentrated in lymph nodes, below our skin and mucosa surfaces, and can identify the enemy by sampling, storing, or processing antigens. Antigens are any substance from outside the body that induces an immune response, such as viruses, bacteria, toxins, or chemicals. Dendritic cells present antigens to T cells (formally called antigen-presenting cells, APCs), which in turn can secrete small proteins called cytokines, and in such a way either activate the T cell response or promote tolerance.

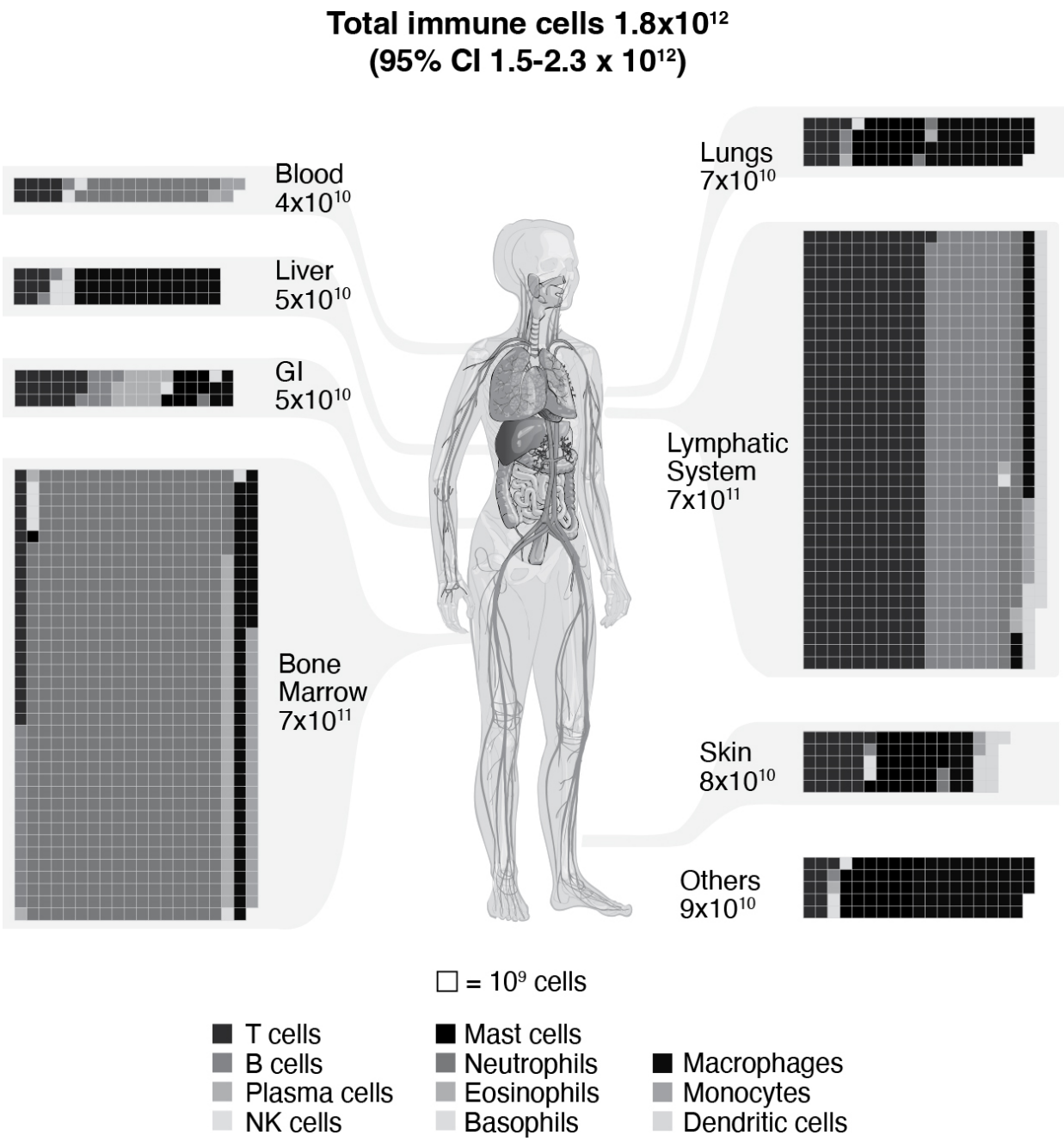


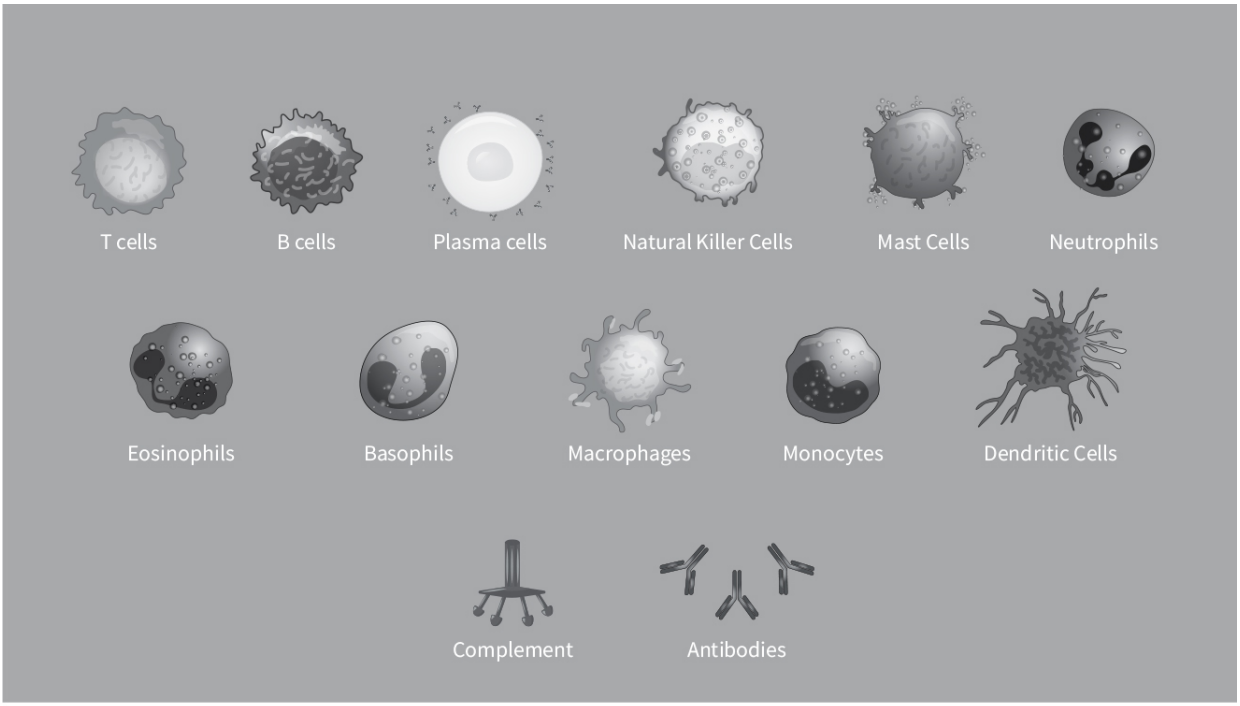

***Figure 9.1.*** The immune cells distribution in our body. Adapted from Ron Sender et al., "The total mass, number, and distribution of immune cells in the human body," *Proceedings of the National Academy of Sciences of the USA* 120, no. 44 (October 2023): e2308511120, https://doi.org/10.1073/pnas.2308511120. Description 45

Tregs are the principal guardian regulatory cells that can put the brakes on, preempting an autoimmune attack and modulating components of both systems. You can think of them as the opposite of immune checkpoint inhibitors, which

take the brakes off cellular immune responses, whereas Tregs can suppress those same kinds of responses. Tregs come to the rescue of a body attacking itself. Even healthy individuals have some autoreactive T cells that circulate in their blood, leading Ruslan Medzhitov and Akiko Iwasaki to propose "Tx cells" that recognize and monitor self-antigens.

Despite the powerful defense our cellular immunity provides, the total population of immune cells accounts for only about 1.2 kg of our bodyweight, with the breakdown by cell type and number shown in the illustration (fig. 9.2). Macrophages, with their large cell size, establish residence in most tissues and account for 10 percent of cells, but disproportionately half of the total mass. Their functional significance to health and disease has been increasingly recognized in recent years, such as their role in the heart, in atherosclerosis, and in inflammatory bowel disease. That is especially the case with microglia, the main macrophage subtype in the central nervous system. Systematic single-cell sequencing of all the different immune cells through the Human Cell Atlas, interactions with cytokines, and with the aging process have markedly improved our understanding of how our immune response operates.

Let me provide a brief sketch for how an autoimmune attack might occur. Let's say a virus, which has an antigen, infects the body. But that antigen is similar to one in the person's body, a molecular mimicry. Dendritic cells detect the infection and activate T cells that connect to both the viral antigen and the self-antigen, with CD8+ killer T cells destroying the cells infected with the virus antigen and the person's cells. At the same time, CD4+ helper T cells can activate B cells to release autoantibodies, which attack one's own proteins. Both the B cells and CD8+ cells evolve to memory B and T cells that can transform the acute response to a chronic autoimmune disease.




## The total population of immune cell types in the body

| Cell type | Total number | 95% CI | Total mass [g] | (95% CI) |
|---|---|---|---|---|
| T cells | $5 \times 10^{11}$ | $3\text{-}7 \times 10^{11}$ | 100 | 60-150 |
| B cells | $3 \times 10^{11}$ | $2\text{-}4 \times 10^{11}$ | 60 | 40-90 |
| Plasma cells | $2 \times 10^{10}$ | $0.5\text{-}6 \times 10^{10}$ | 30 | 4-170 |
| NK cells | $2 \times 10^{10}$ | $0.9\text{-}4 \times 10^{10}$ | 4 | 2-8 |
| Mast cells | $9 \times 10^{10}$ | $6\text{-}14 \times 10^{10}$ | 100 | 40-200 |
| Neutrophils | $7 \times 10^{10}$ | $5\text{-}8 \times 10^{11}$ | 200 | 160-300 |
| Eosinophils | $4 \times 10^{10}$ | $2\text{-}8 \times 10^{10}$ | 10 | 5-20 |
| Basophils | $2 \times 10^{10}$ | $0.4\text{-}7 \times 10^{9}$ | 0.7 | 0.2-3 |
| Macrophages | $2 \times 10^{11}$ | $1.3\text{-}3 \times 10^{11}$ | 600 | 300-1,400 |
| Monocytes | $3 \times 10^{10}$ | $2\text{-}4 \times 10^{10}$ | 13 | 9-20 |
| Dendritic cells | $5 \times 10^{10}$ | $3\text{-}8 \times 10^{10}$ | 100 | 40-300 |
| **Total** | $1.8 \times 10^{12}$ | $1.5\text{-}2.3 \times 10^{12}$ | 1,200 | 800-1,900 |

***Figure 9.2.*** Schematic of immune cell structure (upper) and population of cell types (lower). Adapted from Philipp Dettmer, *Immune* (New York: Penguin Random House, 2021); and Ron Sender et al., "The total

mass, number, and distribution of immune cells in the human body," *Proceedings of the National Academy of Sciences of the USA* 120, no. 44 (October 2023): e2308511120, https://doi.org/10.1073/pnas.2308511120. Description 46

In recent years, we've seen more evidence that autoimmunity is linked to nutrition. Caloric restriction leads to reprogramming of T cells to be anti-inflammatory and promotes the gut microbiome's production of anti-inflammatory metabolites. Fasting activates neurons in the brainstem, with a fortuitous redistribution of T cells to the bone marrow and reduction of cytokine production. That inhibits autoimmunity, while obesity promotes it via adipose tissue, which releases cytokines that cause an overproduction of pro-inflammatory T cells and a decline in protective Tregs. Obesity is a known risk factor for several autoimmune diseases, such as multiple sclerosis and type 1 diabetes. Since energy-sensing pathways are so important in modulating the immune response, the possibility of "pseudo-starvation" with drugs like rapamycin or metformin (mTOR inhibitors) has been raised to simulate caloric restriction's impact on autoimmunity.

A big discovery in 2024, considered a black swan event, was the rheostat circuitry for how the brain can dial up or down our immune response. By injecting bacteria compounds into the abdomen of mice, a brainstem region was activated. The inflammation signals were transmitted via the vagus nerve to the caudate nucleus soltaris. When these neurons were activated, inflammation was reduced by about 70 percent. In contrast, when the neurons were silenced, an immune response was unleashed. Single-cell sequencing further unraveled two discrete circuits, one for pro-inflammatory and the other for anti-inflammatory molecules. In the mouse model of inflammatory bowel disease, manipulating the circuit led to rip-roaring colitis or protection from inflammation. While not immediately clinically transformative, this work lays the foundation for specific neuromodulation of our immune system in the future.

## GENETICS, CHROMOSOMES, AND AGE

Many autoimmune conditions cluster in families, and even more so among identical twins. It isn't uncommon for people to have more than one

autoimmune condition. The genome variants linked to the risk of these conditions have considerable overlap for certain human leukocyte (white blood cell) antigen (HLA) variants, and more than 90 percent are in regulatory regions of the genome (not in genes' protein coding regions). Indeed, single-cell mapping has identified remarkable overlap of likely causative variants across lupus, rheumatoid arthritis, Crohn's disease, multiple sclerosis, ankylosing spondylitis, and type 1 diabetes. Many of the autoimmune genetic risk variants have evolutionary origins, representing adaptive responses to pathogens from more than four thousand years ago.

It is likely that most autoimmune conditions arise from two factors: genetic susceptibility, and a second hit, such as a viral infection with Epstein-Barr (as in multiple sclerosis) or Coxsackie B4 (linked to type 1 diabetes). Multiple environmental factors can also play a stimulatory immune response role, including cigarette smoking (with lasting effects long after cessation), air pollution, pesticides, ultraviolet light, and stress.

Three of four individuals with autoimmune diseases are women, but the explanation for that has been elusive, even though we've known it must have something to do with having two X chromosomes (men have only one). A molecule called Xist randomly inactivates one of the X chromosomes in women, silencing its gene expression. Xist clings like Velcro to its X chromosome, but sometimes it essentially breaks loose, fails to deactivate gene expression, and causes a pernicious immune response. Moreover, Xist has associated proteins that can induce autoantibodies, some of which have been identified in women with lupus, scleroderma, and dermatomyositis. That's only a partial explanation of where autoimmune diseases come from—after all, most people with type 1 diabetes are male, and about 20 percent of autoimmune conditions are found in XY individuals. Sex hormones may play a role too, as has been seen with plasma estradiol concentration correlating with antibodies developed after flu shots.

Although there's heightened risk for autoimmunity in women, they have an advantage over men for a favorable profile of "immune resilience," meaning they have a very good, rapid immune response while also having the ability to control it from overreacting and the following inflammation. From an extensive assessment of adaptive immunity in over forty-eight thousand participants, this

trait of immunocompetence-inflammation balance is associated with longevity, improved survival from COVID-19 or sepsis, and more resistance to severe influenza or HIV infections. It's like a Goldilocks story of just the right capability of fighting off infections while at the same time not having an exuberant inflammatory response that can be deleterious.

With aging, immunosenescence sets in, with a decline in responsiveness in multiple ways: the time it takes, the ability to recognize self, decline in young, naive T cells and the diversity of T cells, decrease in circulating B cells, and weaker antibody production. There is also "inflammaging," a low-grade, chronic inflammation process that may relate to older cells being more likely to secrete cytokines. The extent of these changes varies considerably between individuals with the same chronological age. From a group of 135 healthy adults at different ages, including seventy-two participants aged sixty to ninety-six years, with serial, comprehensive assessment over a nine-year period, an individual's immune age score predicted all-cause mortality. This finding, reinforced by several other reports, reflects how closely our immune system is tied with health span and lifespan. Beyond age per se, using single-sequencing, there has even been the discovery of frailty-specific immune cells from frail, older adults. We also know that our T cell aging, via epigenetic clocks, can be far out of step from our chronological age. After age sixty, about 2 percent of people develop persistent self-directed antibodies against type-1 interferon, which increases their vulnerability to viral infections.

## ROADS TO RESTORING IMMUNE TOLERANCE

A healthy person's immune system is tolerant, ignoring—not attacking—one's own antigens and cells. That's what needs to be restored in people suffering from autoimmune disorders. Multiple initiatives underway will, one way or another, benefit those who are already being treated for autoimmune conditions as well as those at high risk. Since current treatments are lifelong, not curative, and are blunt weapons for nonspecific immunosuppression, the cumulative risk for developing resistance and opportunistic infections is high. The economic burden of current autoimmune treatments accounts for six of the top twenty drugs by

worldwide sales. Despite all that pharmacology, we have not had strategies to *prevent* autoimmune diseases like type 1 diabetes, lupus, multiple sclerosis, and rheumatoid arthritis. With multimodal AI of genomic data, biomarkers, and electronic health records, the ability to identify the high-risk group is becoming possible, building the foundation for prevention.

That all sounds encouraging, but the challenges to restoring durable tolerance without side effects are formidable. There are two different levels of immune tolerance in our body. Central tolerance is based in the thymus, where T cells differentiate and acquire their T cell receptor expression. There are processes of selection in the thymus to weed out cells that might attack one's own tissues, but they're not, by any means, foolproof. Thus, the need for a backup system—peripheral tolerance—referring to all the innate and adaptive immunity mechanisms to protect against self-reactive T cells. That includes deactivating these cells or turning them into Treg cells.

While it would be nice for there to be one magical (auto-) antigen that could fully explain an autoimmune disorder, that's not the case. For one, we don't even know a principal antigen underlying most autoimmune conditions, and there may be many, and they vary from one person to the next. Beyond that, there's the phenomenon of "epitope spreading," whereby more antigens appear on the T cell surface from further activation and differentiation over time. Antigen-specific therapy—dosing people with antigens—that are known to be associated with an autoimmune condition is tricky because that could paradoxically exacerbate the autoimmune response. But clever strategies are yielding very encouraging results.

Nanoparticles top my list. More than two decades ago, Pere Santamaria at the University of Calgary, who suffers from the autoimmune condition of myasthenia gravis, made a groundbreaking discovery with iron oxide nanoparticles loaded with "bait" antigens (fig. 9.3). The bait antigens (from the major histocompatibility complex or MHC) attract T cells, which in turn multiply and develop into Tregs. The process simulates the antigen-presenting cells—dendritic, immature macrophages, and B cells—that have MHC class II molecules to present to T cells. The massive number of Tregs generated in this way can bind and deactivate cells that carry a multitude of antigens. The University of Calgary spun out Pere Santamaria's company, Parvus Therapeutics,

which has signed licensing and collaboration agreements with Genentech and others worth more than $1 billion, and started its first clinical trial with what are now called "navacims" in 2024 for autoimmune hepatitis.

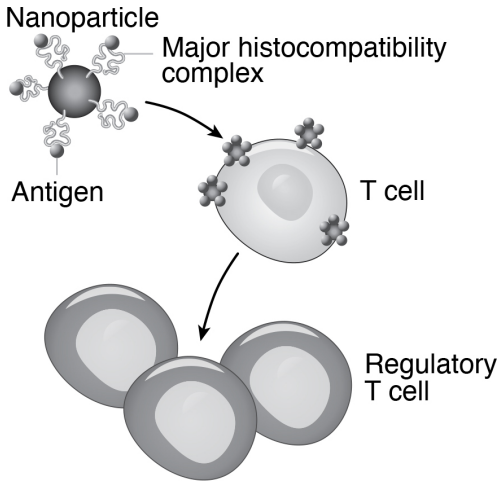



***Figure 9.3.*** "Bait" antigens: nanoparticles carry an antigen from the major histocompatibility complex to attract T cells, which in turn can generate a massive number of Tregs. Adapted from Cassandra Willyard, "Can autoimmune diseases be cured? Scientists see hope at last," *Nature* 625, no. 7996 (January 2024): 646–48, https://doi.org/10.1038/d41586-024-00169-7. Description 47

Speaking of hepatitis, a disease-causing liver inflammation, most people would not have thought a basis for curing autoimmune disease might operate through that organ. But more than a decade ago, a paper was published that portrayed the liver as "a type of 'school' where liver antigen-presenting cells function as 'teachers' and circulating immune cells function as 'students.'" By attaching sugar molecules (glycosylated) to antigens, Jeffrey Hubbell and colleagues at the University of Chicago were able to get the liver antigen-presenting cells to delete autoreactive CD4+ and CD8+ cells and expand Tregs, preventing type 1 diabetes in the experimental mouse model. Subsequently, the same team used this approach with glycosylated myelin proteins in a mouse model of multiple sclerosis, which stopped neural damage and reversed symptoms, and a nonhuman primate model. Their company, Anokion, has completed phase 1 trials in multiple sclerosis and celiac disease and is moving onto phase 2 for these conditions.

In mouse models, there's been success with tolerogenic vaccines for autoimmune encephalomyelitis, the animal model that resembles multiple

sclerosis, and in the preclinical model for rheumatoid arthritis. The former was achieved by an mRNA-based nanoparticle approach by BioNTech, a company behind the COVID mRNA vaccine, using a myelin oligodendrocyte peptide as the antigen. A vaccine comprised of a glycosylated collagen antigen that binds to a T cell receptor was effective in the rheumatoid arthritis model with a potent effect of stimulating Tregs and protecting against arthritis. These are examples of single autoantigen vaccines, but there are many others, such as the use of a cocktail of autoantigens, and ongoing clinical trials for a variety of autoimmune disorders. The term *inverse vaccines*—deactivating the immune response—has gained usage because, historically, vaccines were intended to strengthen our immune system, whereas these and related approaches have the opposite goal.

Our creative genius for cellular engineering is being exercised in other ways. B cell monoclonal antibodies have limited success for autoimmune disease, owing to persistence of auto-directed cells in lymph nodes and other parts of the body. The success of a single infusion of engineered T cells that bind to CD-19, a protein on the surface of all B cells, has been striking. In 2022, the first report of five patients with lupus with full resolution of symptoms was eye-opening, as was the finding that when their B cells remerged, there was no relapse. It was as if the purge of B cells acted as a computer reboot with Control-Alt-Delete. This strategy essentially kills all the B cells (inducing B cell aplasia), with disappearance of all autoantibodies. It subsequently worked exceptionally well in fifteen patients with different systemic autoimmunity (eight patients with severe lupus, four patients with systemic sclerosis, and three patients with autoimmune myositis) with drug-free remission for all during a follow-up mean of fifteen months.[I] The wipeout of B cells would be considered a risk for infection. However, most of the infections in this group have been mild to moderate, involving the upper respiratory tract, including COVID-19. Lawrence Steinman, a leading immunologist who is considered the father of antigen-specific therapy, said, "These results are mind-blowing, boggling, outstanding." Another perspective was offered by Chyi Hsieh, a clinical scientist at Washington University: "I've been doing rheumatology for almost 25 years now, and it's probably the most singular, impressive result that I've seen."

These remarkable results with CD-19 targeting for refractory autoimmune conditions were achieved with the laborious and expensive process of engineering a person's T cells. A much simpler, off-the-shelf approach was used successfully in several patients with autoimmune diseases, paving the way for a more practical strategy to deplete B cells.

A more specific approach has been to target the culprit B cells, such as has been seen with pemphigus, an autoimmune skin disease, with desmoglein as the antigen protein. That specific B cell targeting strategy is the one that Cabaletta Bio is taking for myasthenia gravis and other autoimmune conditions. Many other specific B cell targets are being explored.

But B cell targeting is just one part of the story for engineering cells to restore tolerance; there are many autoimmune conditions for which other cells are the auto-directed drivers. In animal models of asthma, CAR-T targeting eosinophils were protective. Treg engineering has shown promise in multiple autoimmune conditions, with readiness for clinical trials in type 1 diabetes, rheumatoid arthritis, and multiple sclerosis. Sonoma Biotherapeutics is moving ahead in inflammatory bowel disease and rheumatoid arthritis with a targeted Treg approach, such as CAR-reactive citrullinated antigens seen in the latter condition. Instead of the current laborious, time-consuming, and expensive way of manufacturing CAR cells outside the body, Capstan Therapeutics is pursuing an in vivo (inside the body) cell modulation approach with mRNA nanoparticle delivery.

Overall, T cell engineering will likely be more challenging than B cells because their depletion will not be tolerated as well. Nevertheless, a proof of concept was achieved with marked success and without untoward effects in a patient with ankylosing spondylitis with a selective T cell depletion antibody targeting an autoantigen (TRBV9+). This antigen may well be useful for restoring tolerance in psoriatic arthritis, uveitis, and inflammatory bowel disease.

It would not be at all surprising to see genome editing of engineered T cells for facilitating tolerance, as has been done for boosting the immune response against cancer. For all these uses the living drug property of engineered cells must be kept in mind, with the potential for cytokine release syndrome and the downstream consequences of interfering with the immune response over time. That's why

antiaging strategies are being developed for these cells before they're administered to patients. Nonetheless, the cell engineering approaches are collectively showing much promise for promoting tolerance.

But what about some tools from closer to home? We already can successfully transplant a patient's own hematopoietic (blood) stem cells (after depleting lymphocytes [AHSCT]) in the treatment of autoimmune conditions, such as multiple sclerosis, Crohn's disease, lupus, and systemic sclerosis. However, it is a complex strategy that requires two hospital-based stages, the first to mobilize the stem cells over multiple successive days, and the second requiring hospital admission with chemotherapy and immunotherapy to ablate all the lymphocytes (B *and* T cells), followed by reinfusion of the person's collected blood stem cells. Unlike most of the previous approaches reviewed, it lacks specificity, carries substantial up-front risk from the cell ablation therapies, and is unsuitable for a prevention strategy, but, again, it has been associated with some cures. AHSCT is the subject of multiple randomized trials, particularly multiple sclerosis. The potential to engineer blood stem cells within the body, instead of having to remove them and ablate cells, may offer a more practical, lower-risk alternative in the future.

We come to the last on my list of extremely promising approaches to restoring tolerance and curing autoimmune diseases—and it is far from the least. After all, the gut microbiome, with approximately forty trillion microorganisms, is center stage for coordination of our immune response. The gut-associated lymphoid tissue (GALT) is considered the largest immune organ in the body, with the greatest number and diversity of immune compartments and immune cells. Just one cellular component, the intestinal intraepithelial lymphocytes (IELs), one of the largest populations of T cells in the body, has been shown to have a metabolic role affecting GLP-1 levels after eating. The multiple compartments include Peyer's patches in the small intestine, especially in ileum; extensive lymphoid tissue in the appendix; and many lymphoid follicles throughout the small and large intestine. These patches and follicles are in intimate contact with the inside space (lumen) of the gut and have the array of B (naive, memory), T (naive, CD4+, CD8+, Treg), and dendritic cells. Gut cells have been demonstrated to produce complement proteins, and when blocked in an experimental model, they

improved outcomes. The microorganisms produce metabolites, especially short-chain fatty acids, that modulate the function and differentiation of these immune cells. A pro-inflammatory effect, with production of cytokines, can disrupt the intestinal barrier and leak microbes and substances into the blood. There is also the bidirectional gut-brain axis, with the embedded gut (called enteric) nervous system (nicknamed the "second brain") that communicates with the central nervous system through neurotransmitters, the vagus nerve, and ganglia. Reflecting how impactful this axis is is the recent finding that it even regulates our motivation to exercise!

Across a multitude of studies, the gut microbiome composition in people with autoimmune conditions is different from those who are healthy. That doesn't establish cause and effect, but it does suggest fruitful lines of inquiry. In lupus, for example, an *Enterococcus* species (*E. gallinarum*) has been implicated as disease causing, with specific autoantibodies, in susceptible individuals. A strain of *Subdoligranulum* has been implicated to be causal in some individuals with rheumatoid arthritis, likely via gastrointestinal barrier breakdown. The onset of type 1 diabetes has been correlated with an antibody response to certain gut bacteria. In multiple sclerosis, patterns of microbiome alterations have been noted in several studies. Interestingly, a systematic review of eighty-two studies of the gut microbiome in autoimmune disorders and cancer found inversion, in which certain *Bifidobacterium* species are increased in autoimmunity, decreased in cancer. In general, the imbalance of microorganisms in the gut microbiome likely plays a substantial role in autoimmune diseases.

The first rigorous trial to show efficacy against Long COVID tested a microbiome strategy. Long COVID is a condition that is characterized by a dysregulated immune system (gone haywire) with autoimmune features. In Hong Kong, 460 Long COVID patients were randomly assigned to a synbiotic preparation (three lyophilized Bifidobacteria strains and three probiotic compounds) or placebo. Significant improvement of symptoms in the microbiome intervention group was observed through the double-blind design.

From single-cell analytics, the discovery that activated dendritic cells produce lactate, alongside a probiotic that produces lactate, both markedly suppressed T cell–mediated autoimmunity, which may have important implications regarding

manipulating our microbiome. A kidney autoimmune disease, IgA nephropathy, has been linked to a specific gut microbiome, *Akkermansia muciniphila*. In context, the outlook for fine-tuning the gut microbiome with prebiotics, stimulating the growth of specific bacteria, and probiotics, with microorganisms, is exceptionally promising. We also know that response to autoimmune treatment can be affected by the gut microbiome composition, such as seen with the effect of sulfasalazine therapy for inflammatory bowel disease or the response to teplizumab for type 1 diabetes. Caloric restriction has an impact on gut-mediated immunity, promoting large intestine Bifidobacteria, short-chain fatty acid production, and dendritic cell function. A randomized trial testing the addition of navy beans to the diet showed a pronounced shifting of the gut microbiome toward a more diverse, favorable composition with reduced immune and inflammation biomarkers.

The totality of evidence suggests that manipulation of the gut microbiome will evolve to be an important anti-inflammatory or even tolerogenic pathway, be it as an adjunct to one of the many prospects reviewed here or perhaps even as a sole therapy. The manipulation may take the form of probiotics, introduction of particular bacterial strains, fecal transplantation, antibiotics, vaccines, and dietary interventions.

## TOWARD TOLERANCE IN SPECIFIC CONDITIONS

It is a sign of the revolutionary time we live in that the first drug to delay the onset of an autoimmune disease was proved to work and approved by the FDA in 2022. The disease was our nemesis, type 1 diabetes. Since 1999, researchers have been studying how to interrupt T cell attack of beta islet cells of the pancreas, and achieve tolerance. Their drug of focus has been teplizumab, a monoclonal antibody that binds to the surface CD3 receptor specific to T cells. By 2021, a fourteen-day course of the antibody for high-risk relatives was shown, in a randomized trial of seventy-six participants, to significantly delay the onset of diabetes (sixty months versus twenty-seven months for the antibody vs. placebo, respectively) and improve beta cell function. Some individuals had true interruption, without ever developing type 1 diabetes. The FDA approved

teplizumab for prevention of type 1 diabetes for people who had two or more autoantibodies and abnormal glucose metabolism in 2022. Later, in 2023, a phase 3 trial of 217 children and teens newly diagnosed with the disease (in contrast to the high-risk previous trial), given two twelve-day courses of the antibody, had significant improvement of stimulated C-peptide levels, a key metric indicating preserved beta cell function. The mechanism for teplizumab's durable effects appears to be related to promoting CD8+ T cell exhaustion.

That opens the door for more effective or complementary means of preventing this type of diabetes. One direction is toward more specificity of antibodies to T cells that attack insulin-producing cells instead of targeting all effector T cells. An antiviral approach with the combination of pleconaril and ribavirin for new-onset cases was assessed in a randomized trial with success for preserving β-cell function as reflected by C-peptide production. Baricitinib, a potent anti-inflammatory used in rheumatoid arthritis and severe COVID, was tested in a randomized trial also of new-onset cases, and preserved β-cell function. In people aged twelve to twenty-four years with a specific HLA type (DR3-DQ2) associated with type 1 diabetes, autoantibodies (GAD65), and recent onset of the disease, a randomized trial of an intralymphatic autoantigen called GAD-Alum compared with placebo led to improved glycemic control.

A vaccine against Coxsackie, a virus that is strongly associated as a risk factor for type 1 diabetes, has been developed and needs to be evaluated. Promoting tolerance with use of oral, powdered insulin to promote Tregs has some promise. Giving Treg cells failed in a clinical trial of new-onset cases, but the results suggested it was the quality of the cells rather than quantity that was important. The gut microbiome is clearly influential; microbiome manipulation, such as with single strain introduction of *Bifidobacterium* longum, is one of many strategies being assessed. Preventing childhood obesity, another risk factor, is essential. The potential benefit of GLP-1 drugs to help preserve β-cell function, as has been seen with semaglutide in a small group of new-onset diabetes, needs further exploration. Stem cell–derived pancreatic islet cells have successfully eliminated the need to inject insulin in a clinical trial, but the participants had to take immunosuppressant drugs to prevent rejection. Transplantation of stem cell–derived β-cells that are genome edited to avoid rejection would be a more

attractive route and is being pursued for a potential cure. In sum, there is no question that the multiprong strategy for both prevention and "building a bridge to a cure for type 1 diabetes" is underway.

Primary prevention of type 1 diabetes is a bigger challenge than intervening once the condition is manifest with considerable loss of β-cell mass and function. As with all autoimmune conditions, we must find the high-risk people, and at the earliest point possible in their lives. For type 1 diabetes, that can be helped by screening with autoantibodies, since only 15 percent of people who develop this disease have a family history. Some have advocated routine autoantibody screening to be done in children at two points, at ages three to five and eleven to thirteen years, but that hasn't been initiated. Another way to assess risk is via genomics, which would antedate the appearance of autoantibodies if done early, be it polygenic risk scores that have been amply validated across ancestries or whole genome sequencing. Taking a polygenic risk score at birth has been advocated to guide the need for autoantibody testing. Particularly screening antigen-specific CD4 T cells or islet-reactive CD8+ cells could be used to detect high risk at very early stages before autoantibodies are manifest. A natural killer cell gene expression profile has also been shown to identify high-risk individuals. Accordingly, there is no shortage of ways that we can screen, integrate the data with multimodal AI, and be in a far better position to prevent this disease in the future.

Rheumatoid arthritis sufferers have new treatment options too. Abatacept is a fusion protein that works on the antigen-presenting cell (compared to the T cell as in type 1 diabetes with teplizumab), binding to its CD80 and CD86 receptors, to block T cell activation. It had already been shown to be helpful for treatment of rheumatoid arthritis but used in only 5 percent of patients. In a randomized trial of people at high risk, with presence of anti-citrullinated antibodies, joint pain without swelling, and signs of inflammation in MRI scans, all symptoms improved, and benefits persisted after six months of treatment. A second randomized trial that treated for twelve months compared with placebo showed similar results, but over the next twelve months, the efficacy waned. The results of these trials were deemed a substantial improvement over current treatments such as methotrexate and rituximab for autoantibody-positive arthralgia.

This can be seen as a beginning of the work toward restoring tolerance for more definitive treatment, and ultimately prevention. Tolerogenic vaccines, such as one with citrullinated peptides, or one that targets antigen-specific T cells, have very encouraging preclinical data.

As with type 1 diabetes, immune tolerance and preventive strategies for rheumatoid arthritis are being actively pursued, and an autoantibody can be used to identify high-risk individuals. Poor gum health can trigger anti-citrullinated autoantibodies, contributing to disease development in susceptible individuals.

Lupus research has joined the health span revolution. Besides the impressive clinical results for lupus using engineered T cells directed against CD-19, the use of Treg cells with the Smith-specific autoantigen of lupus nephritis stopped disease progression in the experimental model of this severe manifestation of the disease. This could be a launchpad for using Treg cell infusions targeting specific autoantigens playing a role for several autoimmune conditions. Much work has been done to identify causative genes of lupus, such as toll-like receptor-7 (TLR7), and single-cell sequencing to find specific CD8+ (GZMH+) T cells has been markedly expanded in some patients with lupus. Another toll-like receptor (TLR9) found in B cells was shown to impair central B cell tolerance, a novel pathway that could be suppressed.

Again and again, we see one line of research having implications that open up multiple others. A discovery in inflammatory bowel disease of intraepithelial lymphocytes with specific T cell receptor antigens tied to loss of self-recognition is one path to developing immune tolerance in the future. People with celiac disease are stuck with a gluten-free diet for life, which is exceedingly difficult to maintain. Given the central disease-causing role of CD4+ T cells specific for gluten, multiple companies are in clinical trials with modified gluten proteins as antigen-specific therapy. A major genetic discovery underpinning inflammatory bowel disease and likely other autoimmune diseases was the finding of a disease-causing pathway that drives inflammation and appears to be druggable. This could yield a preferred, more focused approach compared with the way we use broad immunosuppression treatments today. Many more therapies are in the pipeline, including Tregs, microbiome intervention, and use of the liver, as previously discussed. While it's just in the mouse model so far, a single infusion

of CAR-T cells provided durable protection against asthma attacks, potentially quashing an autoimmune form of this common disease.

The antibody-drug conjugate strategy we discussed in the cancer chapter is now being tested in clinical trials for autoimmune diseases combining TNF-blocking antibodies with a glucocorticoid (like cortisone) receptor to maximize efficacy. So are bispecific T cell engagers in patients with refractory rheumatoid arthritis.

## THE IMMUNE RESPONSE CONTROLLED

In 2015, I attended a meeting in Boston with George Church, one of the pioneers of genomics and genome editing, to discuss the exciting new frontiers in life science. It stuck with me because he said that it would be possible to edit tens and even hundreds of genes at once, to fully block the recipient's immune response to a transplant organ. This would stimulate the field of xenotransplantation, particularly transplants from pigs, which have strong human resemblance in structure and physiology. Since it was in the early days of CRISPR editing, when successfully targeting one gene was daunting enough, I thought Church's idea was far-fetched. But now we're seeing pig organs with more than sixty genomic edits that knock out the pathways to hyperacute rejection (through three glycan antigens), blocking the complement cascade, reducing the potential for clotting, and inactivating retroviruses that could risk transmission. All the cell and antibody strategies we've discussed for promoting immune tolerance have come into play for organ transplantation, but acute and chronic organ rejection remains a very serious risk. Moreover, the dire shortage of donor organs leaves many people highly vulnerable on waiting lists, or not deemed as candidates for transplantation. Each day in the United States, seventeen people die while waiting for an organ transplant.

Genome editing of pig organs has raced ahead since 2015. The first pig heart, complete with ten modifications to its genes, was transplanted into a patient who had been denied by four heart transplant programs. The same ten genomic edits are being used for kidney transplantation. For these early attempts, the transplant recipients received standard immunosuppressive therapy, and the editing was not

sufficient or intended to achieve immune tolerance. It represents the initial steps of precise and extensive genome editing—to take control of our immune response—for promoting successful xenotransplantation.

In discussing cancer and the control of our immune response, we've covered both ends of a spectrum—from the ways cancer can defeat our immune system to that system's own unbridled, self-directed attacks. While there will always be more to learn about the intricacies of this exceptionally complex system, the progress that is being made across the spectrum deserves our appreciation and kudos. It exemplifies how pivotal our immune system is to every organ's function, our health, and our health span. Just as we're seeing engineering our B and T cells and vaccines help us fight cancer, these same tools are showing exceptional promise for inducing tolerance. Dialing up or down, on demand. That represents new and vital progress; controlling our immune system unlocks vast potential to prevent diseases.

What is the ultimate potential? As we get into advanced age, we'll someday be able to rejuvenate our immune cells to prevent immunosenescence and inflammaging. Hold that thought for a bit.

Meanwhile, there are new threats coming our way. What new roles will our immune system play as we fight off novel infectious pathogens?

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I. CD-19 CAR-T cells have also been used to treat patients with multiple sclerosis, the autoimmune condition we'll get into much more in chapter 10.

# 10

# Defeating Infectious Agents

In the early months of the COVID-19 pandemic, we faced an existential crisis. How quickly we tend to forget the catastrophes of mass deaths, overwhelmed hospitals without enough ventilators that were inundated with very sick patients lying on gurneys in hallways or in their cars in the parking lots. Especially among people in the second half of their lives, who were in fact at high risk, there was a pervasive sense of fear and doom.

By April 1, 2020, just a few months after the threat was recognized, a forecast was made that we might have a COVID vaccine (against SARS-CoV-2) within *eighteen months*. Ridiculous! No vaccine program had ever come close to such a short time frame! The average time from identification of the causal infectious agent to a licensed, commercially available vaccine is eight to ten years, and that's for the ones that were proven safe and effective. Even for mumps, the fastest developed vaccine in history, it took four years. As you can see from the graph below (fig. 10.1), some vaccine programs took over one hundred years, and many, like tuberculosis or human immunodeficiency virus (HIV), are still being worked on. In the year prior to COVID's emergence, Anthony Fauci and colleagues wrote: "In total, 15 to 20 years would be a typical timeframe from virus discovery to vaccine availability if the process proceeds smoothly and there are no major biological or logistical challenges." A realistic projection for a COVID vaccine from several experts was November 2033. That's 2033. A more optimistic note,

based on the average of all FDA-approved vaccines between 2010 and 2020, was 8.1 years of clinical development.

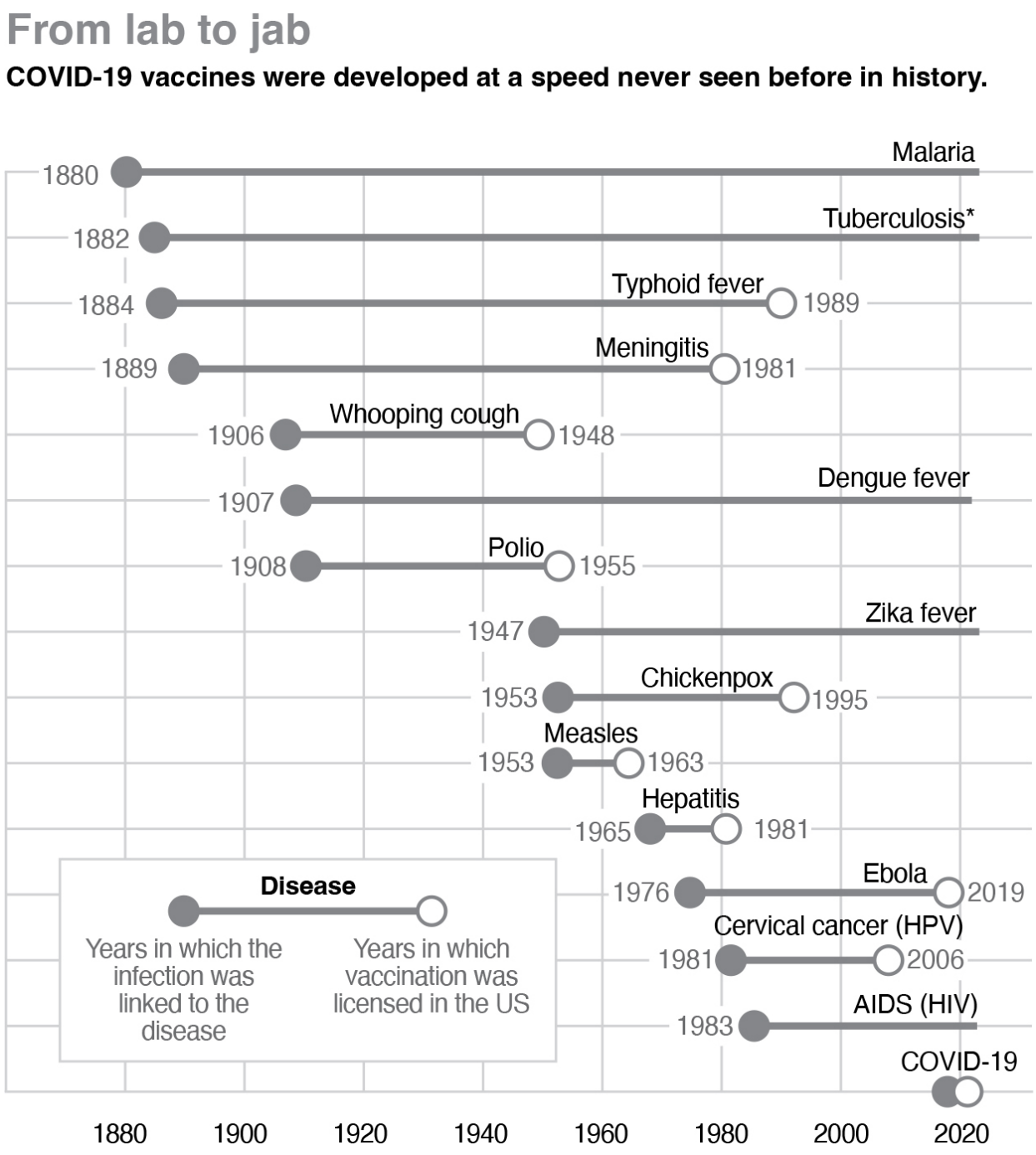



***Figure 10.1.*** Time lag from pathogen identity to developing a successful vaccine, including many without a vaccine (no date at right). Adapted from International Monetary Fund, Communications Department, "Picture this: The journey of the Covid-19 vaccine," *Finance & Development* 58, no. 4 (December 2021): A012, https://doi.org/10.5089/9781513595894.022.A012; and Samantha Vanderslott et al., "Vaccination," Our World in Data, accessed September 5, 2024, https://ourworldindata.org/vaccination. Description 48

It took ten months.

So, what can we learn from the extraordinary success of COVID vaccine programs? It wasn't just getting vaccines with the acceptable threshold of 50 percent efficacy for protection against infections—these came in at 94 to 95 percent reduction. Not surprisingly, multiple factors paved the way. First, the achievement was preceded by more than three decades of work developing mRNA for vaccines. Since messenger RNA codes for proteins, using our body as its own vaccine-production factory was theoretically an attractive alternative. It is a much slower and complex project if the vaccine is made of proteins rather than mRNA, or an attenuated virus that simulates the immune response of having an infection without actually getting one. The hope was that mRNA vaccines would eliminate complicated cell-based production methods, avoid integration with our genome (as could occur with a DNA vaccine), and be less likely to be met with an individual's preexisting antibodies. The scalability for mRNA is huge: a five-liter bioreactor on a lab bench can make nearly a million doses of vaccines!

But for many years it seemed this wouldn't be possible because introducing mRNA into animals led to massive inflammation. The mRNA sequence consists of four letters or nucleotides: adenine, guanine, cytosine, and uracil; a three-nucleotide sequence codes for an amino acid, the backbone of proteins. A breakthrough occurred in 2005, when Katalin Karikó and Drew Weissman discovered that changing the nucleotide of mRNA from uridine to pseudouridine largely preempted the inflammatory response. This discovery, recognized by the 2023 Nobel Prize, ultimately paved the way for the rapid development of mRNA COVID vaccines in 2020.

What protein the mRNA should make once it gets into cells is the next critical challenge. In January 2020, the first sequence of the SARS-CoV-2 virus genome became available. That identified the codons (or specific instructions) for making the spike protein, the business part of the virus that attaches to a host's cells. The science community had been building toward this moment over many years—without realizing it. In 2013, the National Institutes of Health Vaccine Research Center program for respiratory syncytial virus determined the atomic-level structure of the fusion protein that is analogous to SARS-CoV-2's spike protein.

The research showed how stabilizing the fusion protein before it fused to the host cell membrane or entered the cell was key to generating high levels of antibodies that neutralized the virus. By 2015, there had been even more extensive work, using cryo-electron microscopy, to determine the structure of the spike protein of a common cold coronavirus, structurally closely aligned with SARS-CoV-2. That led to a discovery that substituting 2-proline (2-P) amino acids in the prefusion spike protein would induce a far more robust immune response once it got into cells. Accordingly, just one day from having the first SARS-CoV-2 sequence, the design of the vaccine moved forward incorporating this key structure-based 2-P feature. Compressed in that one day was the public health breakthrough result of decades of research.

Substituting things for the way nature built them helped our response to COVID, like using pseudouridine instead of uridine to make mRNA injection in the body noninflammatory, and the two prolines to power up our vaccine-induced immune response. Indeed, much subsequent work has been done with a 6-proline substitution that takes the immune response to an even higher level, although it is not yet incorporated in COVID vaccines.

It wasn't just the mRNA or the spike protein target. When mRNA is injected into the body, it must be packaged so that it's not quickly degraded and maintains its ability to get into cells. For about six decades, in parallel to work with mRNA, there has been relentless pursuit of nanoparticles for efficient delivery. That has culminated in the use of lipid nanoparticles, little bubbles of fat, that wrap the mRNA. The nanoparticles also function as an adjuvant to facilitate the immune response. Each of the four components of these nanoparticles (polyethylene glycol lipid, helper lipid, ionizable lipid, and cholesterol) can and will be improved to reduce the early side effects, ease entry into cells, and promote the durability of the targeted immune response.

One other powerful catalyst of the mRNA breakthroughs was Operation Warp Speed. The idea for this program came from Peter Marks, a senior FDA physician-scientist and Trekkie. A public-private partnership was officially announced in May 2020, funded initially with $10 billion, to accelerate clinical trials, and de-risk mass production of candidate vaccines. That means they would be mass-produced and discarded if the vaccine didn't work in clinical trials, at no

cost to the company that manufactured them. The overarching goal, not lacking any boldness, was to produce and deliver three hundred million doses of safe and effective vaccines by January 2021. Pfizer was not included in the group of companies that were subsidized at "warp speed" but did receive an advance government purchase order in July 2020 for $2 billion. It has been estimated that Operation Warp Speed itself saved the lives of 140,000 Americans.

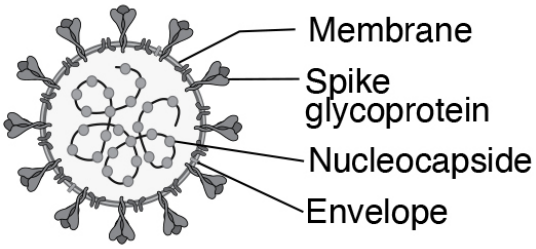
Billions of doses of mRNA COVID vaccines have since been administered to people throughout the world, which has propelled the mRNA/lipid nanoparticle vaccine platform for most infectious diseases, a partial group shown in figure 10.2.

The respiratory syncytial virus program exemplifies the recent extraordinary progress that has been made in vaccines, not relying on mRNA. First identified as a pathogen in 1956, it took sixty-seven years to get a vaccine approved. For context on how desperately one was needed, about 120,000 children die from this virus each year; more than half of these are infants under six months of age. In older adults, in the United States, respiratory syncytial virus accounts for about one hundred thousand hospitalizations, and eight thousand deaths occur each year.

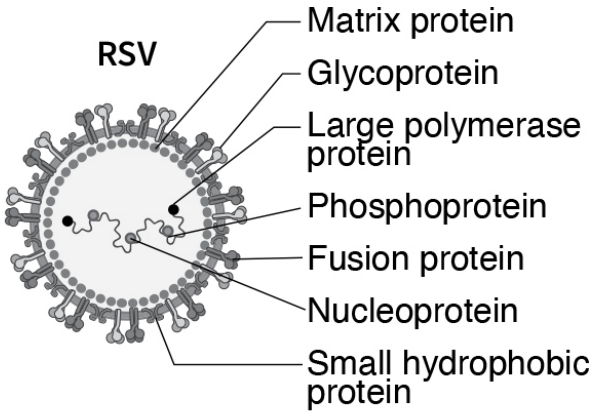
In 2023, the results from four randomized, placebo-controlled clinical trials of three different vaccines were reported. All were single dose with marked efficacy, up to 94 percent protection in people aged sixty and older and 82 percent when given to pregnant women, protecting their babies from RSV out to six months. This remarkable achievement emerged from a rational, structure-based design of a vaccine. Defining the atomic structure of the prefusion protein enabled, ultimately, a powerful immune response in the individual. Again, it is the same winning strategy used for the COVID vaccines, manipulating the spike protein with the 2-proline substitution. Besides the vaccine for protection against respiratory syncytial virus, the first single-dose monoclonal antibody (nirsevimab, Beyfortus) to prevent this virus for newborns and babies was FDA approved in 2023. It, too, is an outgrowth of knowing the prefusion structure, since the antibody binding locks the protein in a prefusion formation, unable to get into cells.

Annual flu shots have limited effectiveness that can vary to as low as 20 percent when there is a mismatch of the flu strain anticipated to that which actually circulates months later. The influenza virus has two main types, A and B, and twenty subtypes; the vaccine primarily targets the surface hemagglutinin (see fig. 10.2), but the virus mutates at a lively rate, and this hampers the vaccine's effectiveness. Imprinting, also known as original antigenic sin, occurs whereby our exposure to preexisting strains of the virus, and the memory B cells that are generated, reduces protection to new circulating strains. Our immune response tends to get stuck on our first exposure to a pathogen, not having the agility to react as adeptly and specifically to a new strain. "Sin," original or not, is perhaps not the best word researchers might have come up with.

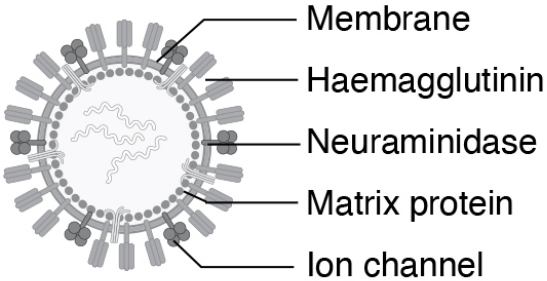
**SARS-CoV-2**



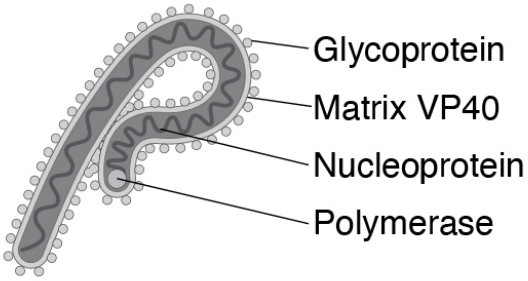

**RSV**



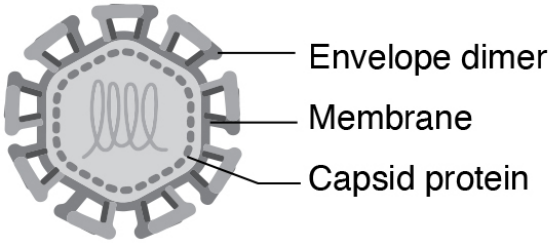

**Influenza virus**



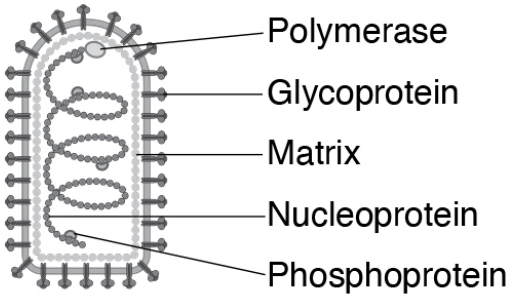

**Ebola virus**



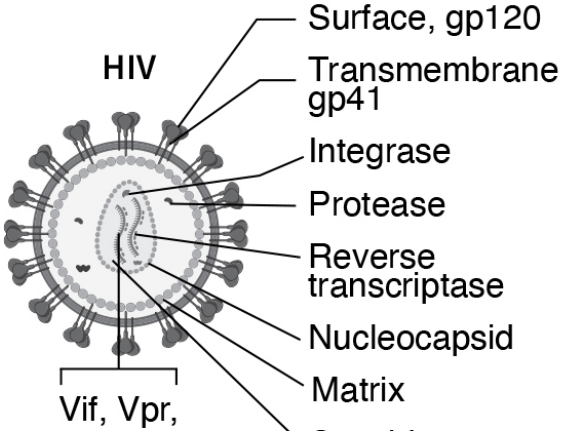

**Zika virus**




**Rabies virus**




**HIV**




***Plasmodium* gametocyte (malaria parasite)**

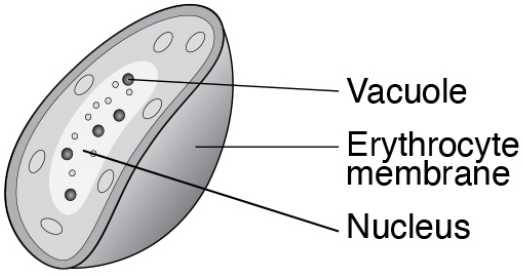


***Figure 10.2.*** Structure of seven different viruses and the malaria parasite. Adapted from Namit Chaudhary et al., "mRNA vaccines for infectious diseases: Principles, delivery and clinical translation," *Nature Reviews Drug Discovery* 20, no. 11 (November 2021): 817–38, https://doi.org/10.1038/s41573-021-00283-5. Description 49

The current flu vaccine is quadrivalent, meaning it only targets two type A and two type B strains. To override these challenges, there has long been the dream of a universal flu vaccine that would target all twenty subtypes of influenza A and B. The mRNA nanoparticle platform has been applied to this objective, with inclusion of twenty encoded antigens targeting each subtype, achieving high neutralizing antibodies and strong protection in multiple experimental models. The attention to atomic-level structure of flu antigens has led to a new ability to generate broad neutralizing antibodies, a path toward a potent and durable influenza vaccine. We're not there yet, but there is intensive effort by several companies, including Moderna, Osivax, Centivax, and Pfizer, who are pursuing a far better universal flu vaccine in ongoing clinical trials.

Structure-based vaccine design is now being used to develop a hepatitis C virus vaccine. The pathogen behind this disease was first identified in 1989, tied to chronic liver disease and liver cancer, killing approximately three hundred thousand people each year of about fifty-eight million people infected around world. But the hepatitis C story isn't just about vaccines. It is also about failing to prevent it. As Francis Collins, the NIH director from 2009 to 2021, mentioned when he wrote about the death of his brother-in-law, we've had curative antiviral pills since 2014. Of the more than 2.4 million Americans who have been infected with hepatitis C, 40 percent don't know it, and 75 percent of infections progress to a chronic form. That's why screening for hepatitis C is essential. The virus leads to slowly progressive scarring of the liver, leading to cirrhosis, liver failure, or liver cancer. Fifteen countries, including Canada, Australia, and Egypt, are on track to eliminate hepatitis C this decade with major national screening and treatment campaigns, but the United States lags far behind. Thus far, not mounting such efforts or squarely addressing the medication's cost and access, the opportunity to eradicate hepatitis C has been squandered.

Pre-COVID, the three biggest infectious disease killers were malaria, tuberculosis (TB), and HIV, and all are being pursued by clinical trials with

mRNA nanoparticles. Malaria was identified as a pathogen in 1880; only in recent times have we started to see successful structure-based vaccines get approved for protection in children, the first vaccine directed against a parasite. Although there are some new drug candidates that we hope will add to the armory in our battle with TB, we're still without a vaccine and have been since the pathogen was identified in 1882. It kills 1.4 million people each year. Now, two different mRNA vaccines against TB are in clinical trials, one of which (M72/ASO1E, Glaxo Smith Kline) is in the final stages and provided 50 percent protection against lung TB in people actively infected.

HIV affects 38 million people, with 1.3 million new infections in 2023. It's resulted in over 600,000 deaths, and lifelong treatment with multiple antiretroviral drugs is required to keep the virus reservoir in check. Over forty years of dedicated effort has not yet delivered a vaccine. Why? HIV attacks and weakens the immune system, giving free rein for the virus to undergo extensive mutation in the body. The virus's genome mutates at the highest rate known to science. Even in one person infected there can be one hundred thousand different strains of HIV, such that a vaccine would have to be effective at inducing neutralizing antibodies against most of these strains. A phase 1 trial of a vaccine of a nanoparticle containing sixty different copies of the HIV envelope protein induced broad neutralizing antibodies against HIV in 97 percent of forty-eight healthy people. Three different HIV mRNA vaccines are being assessed in healthy individuals for antibody production. mRNA nanoparticle vaccines directed to inducing precursor B cells have helped broaden the neutralizing antibody response in mice and nonhuman primates. These are early encouraging steps, but unfortunately, we're still likely many years away from an HIV vaccine. In the meantime, clues from several people with HIV and leukemia who were cured with stem cell transplantation may lead to new approaches that obviate the need for transplants.

The mRNA nanoparticle platform is being applied to improve on traditional rabies vaccines, and is in clinical development for Zika virus, cytomegalovirus, herpes simplex virus, Epstein-Barr virus, Nipah virus, and chikungunya virus. That's just a sample of what seems like a bottomless well of mRNA vaccines with applications beyond infectious disease protection. Cancer vaccines, genome

editing, T cell engineering inside the body (in vivo), and tolerogenic vaccines for prevention of autoimmune diseases are or soon will be realities thanks to this medical technology.

## STRENGTHS AND WEAKNESSES OF THE NEW VACCINES

Initially, the protection against SARS-CoV-2 infection, need for hospitalization, or death was extraordinary—about 95 percent—in the months following mass mRNA vaccination in 2021. Outside the United States, the other vaccine that became available early was an adenovirus platform (made by Astra Zeneca) in the UK, which was also very protective, at approximately 90 percent against severe disease. Breakthrough infections were quite rare, less than 1 percent. It had the look of a home run, maybe even a grand slam, flanked against prior vaccine programs.

But within six months, two things happened. First, the virus evolved into a variant named Delta with significant new mutations in the spike protein that made it more infectious and virulent compared with earlier strains of the virus (the ancestral Wuhan and Alpha). Second, there was waning of immunity with reduction of our neutralizing antibodies and immune response over time. A large placebo-controlled, randomized trial of a Pfizer/BioNTech third shot booster restored protection to 95 percent. The pattern of new variants followed by waning immunity followed by renewed vaccine boosters continued throughout the pandemic. By late 2021, the Omicron variant appeared with more than thirty new amino acid mutations and gathered momentum rapidly, dominating the globe, and the effectiveness of mRNA vaccines against infection plummeted. Vaccine protection against severe COVID remained strong, but even that fell and required successive boosters every six months.

Simply, the mRNA vaccines lacked durability in the face of new variants. Getting an annual flu shot is one inconvenient and undesirable thing; nobody wants to get COVID shots every six months, especially when the side effects may include fever, severe fatigue, headache, and a flu-like illness for at least twenty-

four hours. Even in the first two months after a booster, the ability to block infections was less than 50 percent and fell off quickly.

These deficiencies all have potential solutions. The lack of durability of mRNA shots may be tied to a reduced T cell response from the body's immune system (especially CD8+ cytotoxic cells), which has been noted when compared with the Johnson & Johnson adenoviral vector vaccine. That vaccine, which incorporates double-stranded DNA coding for the spike protein, had to be withdrawn due to rare but serious clotting events—but this problem might be fixed. Either way, self-amplifying RNA vaccines are a new and attractive innovation because a very low dose of mRNA tells the body to make more mRNA. This leads to fewer early side effects and potentially a more durable result than can be seen with the first-generation mRNA COVID vaccines. After a large trial in sixteen thousand participants, Japan approved the first self-amplifying mRNA COVID vaccine in late 2023. It elicited more durable levels of neutralizing antibodies in a head-to-head trial against the Moderna vaccine. Much lower doses of mRNA or more protection and stability afforded by better nanoparticles might also reduce the side effect of myocarditis, a rare but important adverse effect of these vaccines predominantly seen in younger men. Chemical modification of mRNA can give it much enhanced staying power, lasting two to three times longer and boosting activity levels in cells by up to twentyfold. This has been achieved by adding multiple tails to the mRNA, giving it a branching structure instead of its current linear form. Another modification has been to make "nanocages," assembling four receptor-binding domains (part of the spike protein) in a quartet to markedly strengthen the immune response. There are just a couple of examples of how mRNA can be made more potent, specific, and durable. It builds on the prior ways we've manipulated mRNA with the pseudouridine and 2-proline substitutions that helped usher in its clinical success. These are ways to take mRNA to the next level, and that's apart from what we can do with nanoparticles. Beyond mRNA and nanoparticles, what the vaccine is mixed in—the adjuvant—can further strengthen the immune response.

But this will not get us to a universal vaccine that protects against all SARS-CoV-2 variants. That's because the mRNA shots are spike directed, whereas the virus, via natural selection, picks up abundant and diverse spike mutations that

enable it to evade our immune response. The pressure from vaccinations on the virus to evolve sets up a vicious loop. Moreover, in an immunocompromised individual, evolution can accelerate in the body, which likely accounts for the hypermutated Omicron and subsequent "Omicron-like" variants with some thirty new spike protein mutations. Updating a new booster to match the variant doesn't work very well, even with the speed for making updated mRNA vaccines. It takes three months to mass-produce a new booster, and by that time, a different variant typically predominates. Since the SARS-CoV-2 virus has thirty thousand letters, the number of possible variants is essentially infinite. But a vaccine that works against all members of the sarbecovirus or beta-coronavirus family would do the job.

Candidates for a pan-coronavirus "dream" vaccine target other parts of the virus shared by all variants, or they use broad neutralizing antibodies from patients. Such antibodies identify a template for a vaccine that induces their production; in experimental models, these vaccines have been shown to work against diverse beta-coronavirus strains.

However, wouldn't it make more sense to stop the infection as it enters the body, before it spreads throughout the body? Respiratory viruses enter the body via the lining of our upper airway, invading epithelial cells in the nose and mouth. To provide protection here, we need a nasal spray or inhaled vaccine. Mucosal immunity relies on inducing higher levels of a different antibody (secretory IgA) than shots (IgG); as expected, multiple experimental studies have shown superior protection against infection with nasal vaccines compared with shots.

Beyond the lessons from the vaccines, the pandemic overhauled the way we track pathogens. Rather than only tallying the number of people with infections, hospitalizations, and deaths, we now have adopted new methods that provide precise information that anticipates the virus's evolutionary path and burden. Genomic sequencing of the virus from people infected tells us about new variants, when and where they appear, and how quickly they are growing in the new cases. We can track the danger specific variants pose, much earlier than in the past.

At the same time, municipal wastewater levels of the virus foretell the future, at the local level, by many days to weeks before seeing the spread of infections.

Sequencing the virus excreted in wastewater is also a way to detect new variants once an epidemic has erupted. Neither genomic nor wastewater surveillance were used routinely before COVID-19 hit, but now they not only monitor this virus but also are being used for an increasing array of pathogens including mpox, polio, influenza A and B, RSV, norovirus, and more.

These are vital components of modern pandemic surveillance, but significant improvements lie ahead. We know that many infections are asymptomatic, as we've seen with SARS-CoV-2 at least 30 percent—most people are unaware that polio is without symptoms in more than half of people infected. By only tracking people with symptoms, it's a big miss for the true toll of infections and the paths they take in the population as they spread. Further, the exclusive attention to acute infection events ignores the longer-term sequelae. We know that post-virus syndromes can be enduring and disabling, as seen in tens of millions of people with Long COVID, but they have never been systematically tallied. Pandemic surveillance of the future will be able to give real-time risk assessment at the individual and local levels that incorporates all the relevant metrics on a smartphone app—now possible with multimodal AI. The use of the smartphone to record a forced cough, use AI to make an accurate diagnosis of COVID, and differentiate it from other respiratory pathogens, including tuberculosis, is getting more attention, with results exceeding expectations. If further validated, that could help improve speed and reduce health inequities for diagnosis.

If there's one success story for a vaccine changing the face of a dreaded disease, it's the human papilloma virus (HPV) and cervical cancer. Nearly all (99.7%) cases of cervical cancer are attributed to chronic HPV infection. HPV infections enter the genital mucosa surfaces and only low levels of antibodies are formed, which takes more than eight months. Two or three sequential HPV vaccine shots achieve sterilizing immunity—preventing entry of the virus into cells, preventing infections for what may well be the lifetime of the individual. This is "superhuman immunity" because it defies the adage that natural infections induce better immunity than vaccines. It's not the only example; similar vaccine-induced immune response superiority over natural infections is seen with tetanus, Hemophilus influenza type b, and varicella zoster (which can lead to shingles).

The clinical impact of HPV vaccination is nothing short of remarkable. The first national cohort to monitor the long-term outcome of vaccination was Scotland, where it became routine in 2008 with nearly 90 percent of girls aged twelve to thirteen getting at least one dose of the three-dose protocol. How many cases of cervical cancer in these forty thousand women were later found? None. Zero! Even older girls greatly benefited from the vaccination, with a 62 percent reduction from 8.4 per 100,000 women in unvaccinated to 3.2 cases per 100,000 women who received the full vaccine protocol.

Back in the United States, the HPV vaccination rate remains alarmingly low, at approximately 60 percent. Meanwhile, cervical cancer kills more than three hundred thousand women each year. Worldwide, only one in five have had the vaccine. Head and neck cancers, particularly involving the throat, are the most common malignancy caused by HPV. Those cancers have an even higher number of cases per year than cervical, and the vaccine is FDA approved and highly effective for yet another type of cancer. Men can benefit by marked reduction of head and neck cancers, but their uptake, aged twenty-seven to forty-five years of age, is less than one-third of women. Here we have the prototypic superhuman immunity vaccine, which is exceptionally safe at preventing HPV cancers, but the familiar and daunting problem of inadequate population vaccination that could lead to their eradication. That is a problem we cannot ignore.

## VACCINES FOR EPSTEIN-BARR VIRUS, CANCER, AND MULTIPLE SCLEROSIS?

If an infection causes a disease, it makes sense that there could be a vaccine for it. Some diseases long thought to be the result of something other than infection now seem to be falling into the infectious disease category. Maybe even some kinds of cancers.

More than sixty years ago, the Epstein-Barr virus was the first demonstration of a cause and effect for a virus to induce cancer, specifically Burkitt's lymphoma. By electron microscopy, the virus could be seen replicating in the tumor cells. But 95 percent of the world's population has a lifelong infection from this virus

without symptoms, the most common persistent viral infection in humans. Epstein-Barr preferentially infects B cells, and, in culture, the virus induces unbridled B cell proliferation, a reflection of its malignant potential. But an additional factor beyond the Epstein-Barr virus infection appears to be required for development of lymphoma (a chromosome rearrangement) and nasopharyngeal carcinoma (an epigenetic event). Less is known about what factor accounts for this virus's link to stomach cancer. Given that, globally, Epstein-Barr virus is involved in 1.5 percent of cancers, we may be waiting some time for sufficient research to produce a vaccine.

For many years there was a potential link between Epstein-Barr and multiple sclerosis (MS). MS, an autoimmune disease affecting nearly two million people, is more frequent among people who have infectious mononucleosis, often caused by Epstein-Barr. Some pathology tissue postmortem samples from people who had MS contained Epstein-Barr virus. And tellingly, individuals with MS have been seen to have increased levels of antigens directed against Epstein-Barr virus. In 2022, a major report from over ten million young adults in the US military advanced the case for a causal relationship between the two maladies.

From sixty-two million blood samples obtained over a twenty-year period (1993–2013), the timing of Epstein-Barr infection could be determined, correlated with the diagnosis of MS that occurred in 955 individuals, and compared with controls who did not develop MS. The risk of MS increased thirty-two-fold after Epstein-Barr infection, was not associated with any other viruses, and a biomarker for nerve cell injury (serum neurofilament light chain) increased after the Epstein-Barr infection. The conclusion was that this common virus was "the leading cause of MS."

Subsequent reports cemented the case. In the cerebrospinal fluid from patients with MS, antibodies to Epstein-Barr nuclear antigens were found. Molecular mimicry has been a leading proposed mechanism, supported by the finding that a Glial CAM protein in nerve tissue or alpha-crystallin B, expressed by oligodendrocytes, looks like an Epstein-Barr nuclear antigen to the body's immune system. So, the person's immune response to Epstein-Barr infection is to direct antibodies to it, but these antibodies attack neurons too, because of the look-alike proteins. This lays the foundation for a self-perpetuating

autoimmunity loop. In the early phase at initial diagnosis of MS, T cells directed to the Epstein-Barr-infected B cells are found in the cerebrospinal fluid.

Autoimmunity to the central nervous system—brain and spinal cord—and reactive inflammation to the specific attack on myelin, the fatty tissue that insulates neurons, produced by oligodendrocytes, leads to cell destruction and tissue damage. The course of the disease in an individual is generally either a relapsing-remitting form, which usually responds well to current therapies, or the less common progressive form, in 15 percent of patients. The latter, far more aggressive type has been characterized using single-cell sequencing and spatiotemporal mapping, defining the four stages of lesion formation.

Depletion of B cells with ocrelizumab, a monoclonal antibody directed against CD20 surface receptor, steroids, and other immunosuppressive agents, is the most common treatment prescribed. But engineered T cells, including targeting CD19 (as previously reviewed for lupus and other autoimmune diseases in chapter 9), conjugated antibodies, and stem cell treatments are in clinical trials, as are various ways to manipulate the gut microbiome, which has been implicated in multiple studies.

Establishing Epstein-Barr as the most common cause of MS prompted initiatives to develop a vaccine. In the mouse experimental model of autoimmune encephalomyelitis, which mimics human MS, a vaccine that promotes immune tolerance by blocking self-directed T cell response has suppressed the disease. But the main pathway for vaccines has been to target glycoprotein 350 (gp 350), which enables the entry of Epstein-Barr into B cells, and in the case of related cancers, epithelial cells. Another such viral protein (gH/gL/gp 42) has also been targeted for a bivalent (both proteins) vaccine and exhibited strong efficacy in the mouse model. A vaccine study in mice that targeted both gp350 and a multiprotein strategy induced strong antibody and cellular immunity.

Those are some examples being tested in animal models, but two vaccines are already into clinical programs. Both target gp350. The Moderna program is testing the vaccine in healthy adults aged eighteen to thirty years who are Epstein-Barr negative by blood testing. The National Institutes of Health vaccine uses an iron nanoparticle and is a three-dose strategy in participants testing positive or negative.

These trials are intended to demonstrate the inability of the virus to get into B cells as well as the safety of the vaccines. That's altogether different from preventing MS. Since MS is uncommon in people newly infected with Epstein-Barr, and since MS takes years to develop, it will take a long time and very large clinical trials to determine preventive efficacy.

That's where all the progress on genetic susceptibility can come into play. Certain immune system genetic markers are linked to an increased risk of MS, such as HLA-DRB1*15:01, which denotes a greater than threefold risk in whites, as do other HLA markers in African American and Asian populations. Genetic susceptibility has been studied extensively with development of a polygenic risk score based on hundreds of both common and rare genomic variants. Individuals in the highest polygenic risk quartile have about a 40 percent chance of being diagnosed with MS compared with less than 5 percent in the lowest quartile. The list of genomic variants linked to MS keeps growing, such as those responsible for binding in the vitamin D receptor. With extensive assessment of immune markers and cell function, people with up to 260-fold increased risk of developing MS could be identified. A panel of autoantibodies can be used to identify high-risk individuals years before a first attack. Multimodal AI, with inclusion of all electronic health records, labs, images, unstructured notes, autoantibodies, gut microbiome, and environmental and lifestyle+ factors, has begun to unravel MS risk and its arc of progression.

By selecting very high-risk individuals without prior Epstein-Barr infection for an MS vaccine program, we should be able to determine how early in a person's life we might be able to prevent this disease. A parallel approach could also someday be applied to Epstein-Barr-related cancers. To date, the vaccines are not completely blocking Epstein-Barr infections but instead are blocking the ability of the virus to set up camp in B cells. Totally blocking these infections may yield higher efficacy against MS and related cancers.

Additional benefits to such a vaccine seem likely to accrue for other autoimmune diseases such as lupus and rheumatoid arthritis. The body of evidence that has accumulated for Epstein-Barr as the root cause of MS has transformed our approach, and perhaps we'll see a success story like HPV and cervical cancer in the years ahead.

The remarkable progress in our efforts to defeat infectious diseases goes beyond vaccines. Measles, which can be fatal in children, with increasingly occurring outbreaks because of lapses of vaccination, has never had a specific treatment. But a discovery of a potent neutralizing antibody against this virus's ability to get into cells, and potentially other related viruses, is a major step forward. Likewise, prion diseases, such as Creutzfeldt-Jakob disease, which stem from transmissible misfolded proteins, are rare, fatal, devastating neurodegenerative conditions without any effective treatment. An epigenetic editor that induces programmable methylation was able to durably silence expression of prion proteins by more than 80 percent across the entire brain in the mouse model. No less switch itself off once the gene silencing work was done. This innovative work can be seen as encouraging for a set of previously imponderable diseases.

## ANTIMICROBIAL RESISTANCE

Microbial resistance to antibiotics now appears to be as big a global health problem as HIV and malaria. Estimates for annual deaths from this resistance exceed five million. This global health emergency is at least in part an outgrowth of our mass overuse of antibiotics in people and animals. The seven leading bacterial pathogen–resistant killers are *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*. The climate change crisis, with its spread of drug-resistant infections, and the pervasive presence of microplastics, which provide residence for viruses that help spread antibiotic resistance genes, are making this situation even more desperate. Unfortunately, owing to lack of financial incentives, the pharmaceutical industry has largely abandoned discovery and development of new antibiotics to override resistance of the most worrisome pathogens. The last entirely new structural class of antibiotics was discovered in the 1980s. Although about 180 countries have developed national action plans to address antibiotic resistance, which includes routine surveillance measures of sampling the environment, wastewater, the food chain, and human and animal populations, fewer than forty have funded or

implemented them. The AMR Action fund was established as a private-public partnership with twenty-four companies chipping in $1 billion to bring two to four new antibiotics to market by 2030.

While that fund is a start, we face the prospect of ten million deaths from antibiotic resistance by 2050, according to the World Health Organization. Thanks to academic labs, using AI and structure-based discovery, we are seeing a jump forward in promising candidates that are going into clinical trials. The table below summarizes recent discovery of potent molecules against multiple species of concern, using different AI models and structure-based drug discovery.

| Overcoming Antibiotic Resistant Species | Method | Number of Compounds Screened | Citation |
|---|---|---|---|
| C. difficile; M. tuberculosis; A. baumannii; Enterobacteriacae | Deep neural network | >107M – >23 – >8 | Stokes J, *Cell*, February 20, 2020 |
| Burkholderia cenocepacia | Deep neural network | >225K – >43 – >5 | Rahman A, *PLoS Comp Biol*, October 13, 2022 |
| Staph aureus; *E. coli*; Pseudomonas aeruginosa | Structure-based drug discovery | NA | Wu K, *Science*, February 16, 2024 |
| Staph aureus | Graph neural network | >12M – >283 – >4 | Wong F, *Nature*, December 20, 2023 |
| A. baumannii | Generative AI | >13K – >58 – >6 | Swanson K, *Nature*, March 22, 2024 |
| *E. coli*, K. pneumonaie | Structure-based drug discovery | NA | Huseby D, *PNAS*, April 5, 2024 |

Source: compiled by author (original)

MIT researchers discovered Halicin, a drug initially developed to treat diabetes but later found to have antibiotic properties, and related molecules using deep-learning AI. They assessed over 107 million molecules, whittling this down to 23, and identified 8 with potent activity against several pathogens, some verified with experimental models. A similar approach was applied to find an effective antibiotic against *Burkholderia cenocepacia* from over 225,000 molecules, with five compounds having very strong activity. The first new structural class of antibiotics in over three decades came from a graph neural

network, a type of AI. How it worked, starting with over twelve million compounds to get to four nontoxic effective ones, was clearly not simple (these compounds were nontoxic in animal models against methicillin-resistant *Staphylococcus aureus*). A hunt for an antibiotic to *A. baumannii* was the first to use generative AI, ingesting data from over thirteen thousand molecules to get to six with considerable activity. The beauty of this work is that it focused on easy-to-synthesize small molecules, which would have practical advantages for mass production at low cost. AI has also enabled new antibiotic peptide discovery at scale by mining the proteomes of extinct organisms (the "extinctome"!) and from the global microbiome public database. Both efforts predicted tens of thousands of candidate antimicrobial peptides, for which initial assessment of more than one hundred validated them to have functional activity against bacteria. Large-scale screening of drugs that are not antibiotics has also been undertaken, yielding many candidate molecules with potent activity. A large language model like ChatGPT was used to reengineer an existing antibiotic to kill a multidrug-resistant, toxic bacterium.

A very different approach, relying on not AI but traditional structural biology to inform drug design, led to discovery of cresomycin, which has very broad action against several resistant strains of bacteria. A vaccine directed against methicillin-resistant *S. aureus* represents yet another potential approach. So does the first "smart" antibiotic developed that spares the gut microbiome, lolamicin. It works against the genome sequence of disease-causing bacteria and doesn't disturb the friendly, non-disease-causing bacteria that inhabit our gut. Lolamicin is a Gram-negative-specific antibiotic that was shown to be active against 130 multidrug-resistant clinical isolates, and effective in multiple mouse models. Many of these efforts have led to clinical trials with hopes they will prove effective and safe as well as help enrich our armamentarium in the battle against resistant microbes.

Besides AI and structure-based discovery, bacteriophage (phage) therapy offers another highway to outflanking microbial resistance. These are viruses that are the natural predators of bacteria with a representative structure (fig. 10.3) from one of the three families (*Siphoviridae*, shown; *Myoviridae*; and *Podoviridae*). The capsid holds and protects the phage's DNA. The tails help penetrate specific

strains of bacteria. Once inside the bacteria cells, the phage hijacks its resources, rapidly proliferates, and explodes. With that destruction, the proliferation of new phages is released to infect more cells, which occurs exponentially. Bacteria are not defenseless against phages. They have multiple CRISPR-Cas endonucleases and more than one hundred newly discovered defense systems, and the phages have anti-CRISPR counter defenses.

Their abundance in nature is extraordinary, with about $10^{31}$ phages in the biosphere, the most abundant and diverse biological entity on the planet, with $10^{23}$ infections per second on a global scale. The history of phages is long, with their discovery dating to 1896, and their origins more than three billion years ago. Nevertheless, their therapeutic efficacy in combination with antibiotics or as solo therapy for infectious diseases has not been well established. Slowness in the field may be related to obstacles such as a bias against using a *virus* to treat infections, questions on patentability, and pessimistic expectations for regulatory approval.

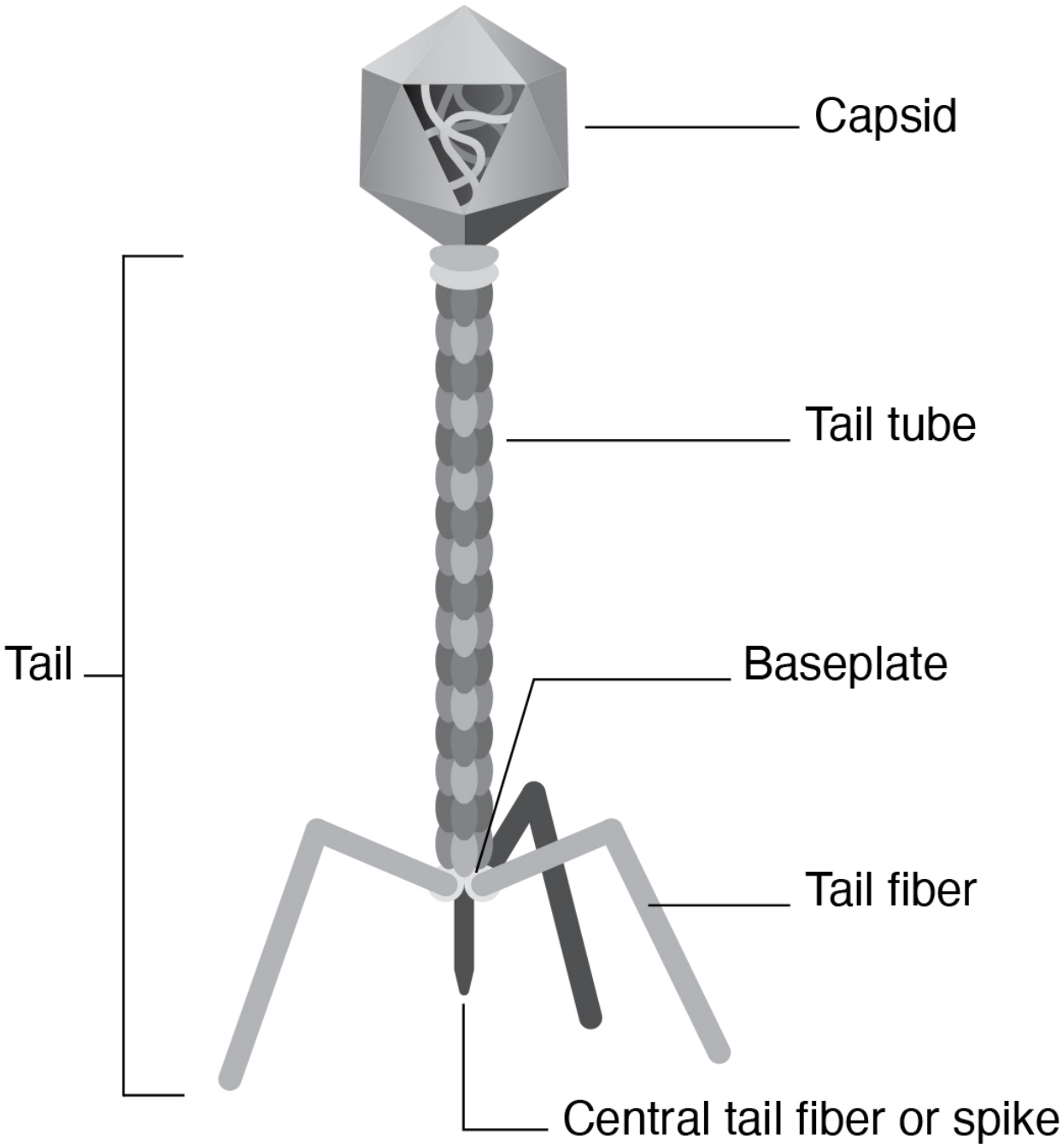



***Figure 10.3.*** Structure of a bacteriophage (phage). Adapted from Franklin Nobrega et al., "Targeting mechanisms of tailed bacteriophages," *Nature Reviews Microbiology* 16, no. 12 (December 2018): 760–73, https://doi.org/10.1038/s41579-018-0070-8. Description 50

That's changing now. With a series of successful case reports for treating life-threatening conditions with multidrug resistance, interest is beginning to soar in recent years, with many more ongoing clinical trials tackling highly challenging bacterial infections that are resistant to antibiotics: *Staphylococcus aureus* for

diabetic foot ulcers, chronic sinusitis, resistant ear infections (otitis media), sepsis, and endocarditis; *Pseudomonas aeruginosa* for lung infection, otitis media, artificial joint infection, osteomyelitis, cystic fibrosis bronchiectasis, and aortic grafts; *Klebsiella pneumoniae* (carbapenem-resistant) for artificial joint infection, gut colonization, and sepsis; *Escherichia coli* for urinary tract infections; and *Acinetobacter baumannii* for pneumonia. Of more than ninety clinical phage trials worldwide, about half are in the United States. Doctors can use the Center for Phage Applications and Therapeutics to find the right phage for the right patient. A compilation of one hundred consecutive cases of personalized phage therapy from a consortium of thirty-five hospitals in twelve countries demonstrated clinical improvement for over 70 percent of patients and eradication of the target bacteria in more than 60 percent. The best results were obtained with concomitant use of antibiotics, but interpretation is limited without a control group. Still, the potential for bacteriophages to aid the fight against drug-resistant bacteria is promising.

All these trials are using natural phages, but the next phase is getting started with multiphage cocktails, phage genome engineering, and synthetic phages. While I have emphasized the use of phages to overcome microbial resistance, there are many potential applications for noninfectious diseases. Examples include phages to treat *Klebsiella pneumoniae* or *Escherichia coli* in inflammatory bowel disease, *Enterococcus faecalis* in alcoholic hepatitis, to manipulate the gut microbiome to improve immunotherapy for colon cancer, and as a vector to deliver drugs or vaccines. The successful use of phage delivery of CRISPR base editors to alter the sequence of *Escherichia coli* and *Klebsiella pneumoniae* in the mouse gut microbiome bodes well for both a future application of phage and another way of overriding antimicrobial resistance.

Sometimes sepsis, an extreme reaction to some infection, kills due to antimicrobial resistance, but mostly treatment failures are from missing the diagnosis or suboptimal medical care. With nearly 50 million cases and 11 million deaths each year around the world, and 350,000 deaths in the United States, this life-threatening, organ dysfunctional syndrome is the third-leading cause of death in US hospitals. Can technology help here? An AI tool was widely adopted throughout the United States to predict the risk of sepsis, but subsequent

assessment showed its performance was poor. This was ascribed to the phenomenon of dataset shift, whereby a mismatch occurs from the dataset a model was developed from compared with the one on which it was deployed. Since that failure was diagnosed in 2021, there have been improvements in AI detection of sepsis that significantly reduced time for starting antibiotic therapy and were associated with an 18 percent reduction in deaths when physicians responded to alerts promptly. In 2024, the FDA approved the first AI model that predicts the risk of sepsis by Presnosis, a sepsis immunoscore that uses twenty-two clinical and laboratory parameters for likelihood of developing this condition within twenty-four hours.

Accurate and timely detection of hospital-acquired infections from central venous access or urinary catheters was fostered by two large language model AIs (GPT-4 and Mistral). Biomarkers and whole-blood gene expression that help make the diagnosis or partition high-risk patients for developing sepsis are also being validated, and they would add to the current algorithms that integrate the patient's electronic health record, labs, and vital signs. It's fair to say that we still have a long way to go to get morbidity and mortality down from sepsis, but use of AI and omics may contribute. There's another strategy that is rarely used but could make a big difference thanks to the latest technology—sequencing the blood or body fluid.

In 2014, Sharon Peacock, a professor of microbiology at the University of Cambridge, wrote, "Microbial sequencing should be done as close to the patient as possible." More than a decade later, despite remarkable advances in low-cost, rapid sequencing, this is only done in a few hospitals around the world. This approach is known as metagenomics, a comprehensive sequencing analysis of everything within a sample. It is an unbiased, direct way of determining the pathogen in a patient, be it bacteria, virus, fungus, or parasite. Not only can the microbe be identified within hours but the sequence can tell us whether resistance to therapy is likely and provide guidance for appropriate antibiotics. Surprisingly, since 2014, with the diagnosis of neuroleptospirosis using metagenomics to save a fourteen-year-old boy's life, this technology has been known but collecting dust. A report in the *Wall Street Journal* in 2023 captured

the virtue of this diagnostic approach with the catchy headline "even brain-eating amoeba can't hide from this cutting-edge diagnosis technology."

The traditional approach, what I've called a "culture of blood cultures" entails getting multiple blood samples and other relevant body fluids (such as sputum, stool, urine, and cerebrospinal fluid) sent for culture, which takes a few days to read out, and even longer to determine antibiotic resistance. In the meantime, the patient gets "empiric" antibiotic therapy, which typically consists of multiple potent, broad-spectrum antibiotics, which carry toxicities, and may not even provide coverage against the offending pathogen. For example, it could be a fungus or virus, not a bacteria culprit, or a bacterium that's resistant to the empiric antibiotics selected. Some pathogens are very slow growing in culture, taking several days to show up. Yet we are stuck in this culture of doing cultures.

Metagenomics is also known as "shotgun" sequencing, and you may be wondering a bit on how it's done. As you can see from figure 10.4, the sample undergoes extraction for short DNA fragments and rapid sequencing (at a cost of $150–$250), which leads to a bunch (about one hundred million snippets) of unclassified DNA sequences. Of these, about 99 percent are human; the nonhuman snippets are the ones of interest. Separating out contaminants and bystanders can be a challenge, but automated interpretation tools help get that done. With the support of cloud-based algorithms of libraries for the gamut of pathogens, and AI, identification of the culprit is made.

In 2020, the University of California, San Francisco, published data on diagnosing unknown infections in 160 patients. With a turnaround time of less than six hours, using nanopore sequencing, compared with traditional culture methodology, there was highly accurate diagnosis of bacteria and fungi pathogens (sensitivity of 79% and 91%, for bacteria and fungi, respectively; specificity 91% and 89%, respectively). In London, among thirty-four patients with a breathing tube in the intensive care unit, within eight hours, metagenomics of sputum samples made the diagnosis in all but one patient (92%). Many cases that were culture negative (no growth in culture) were diagnosed with metagenomics. In Shanghai, in ninety-nine patients, compared with blood cultures, metagenomics made the diagnosis in sixty-five patients versus only thirteen by culture; the other patients had no proof of an infection. A more recent report from multiple centers

in France with over two hundred patients also had a very high yield with rapid sequencing. These studies point to the complementarity of sequencing and culture approaches, but the speed and accuracy of metagenomics gets to the pathogen diagnosis and right treatment days faster. Sending out the patient sample to companies that can do this results in multiple-day delays that approach the time it takes for results from blood cultures to come back. Beyond metagenomics, there are also ultra-rapid peptide tests to diagnose sepsis and assess antimicrobial resistance, also preempting the need for blood cultures. What can't be emphasized enough is that time to the proper treatment is the critical determinant of outcomes for patients with sepsis.

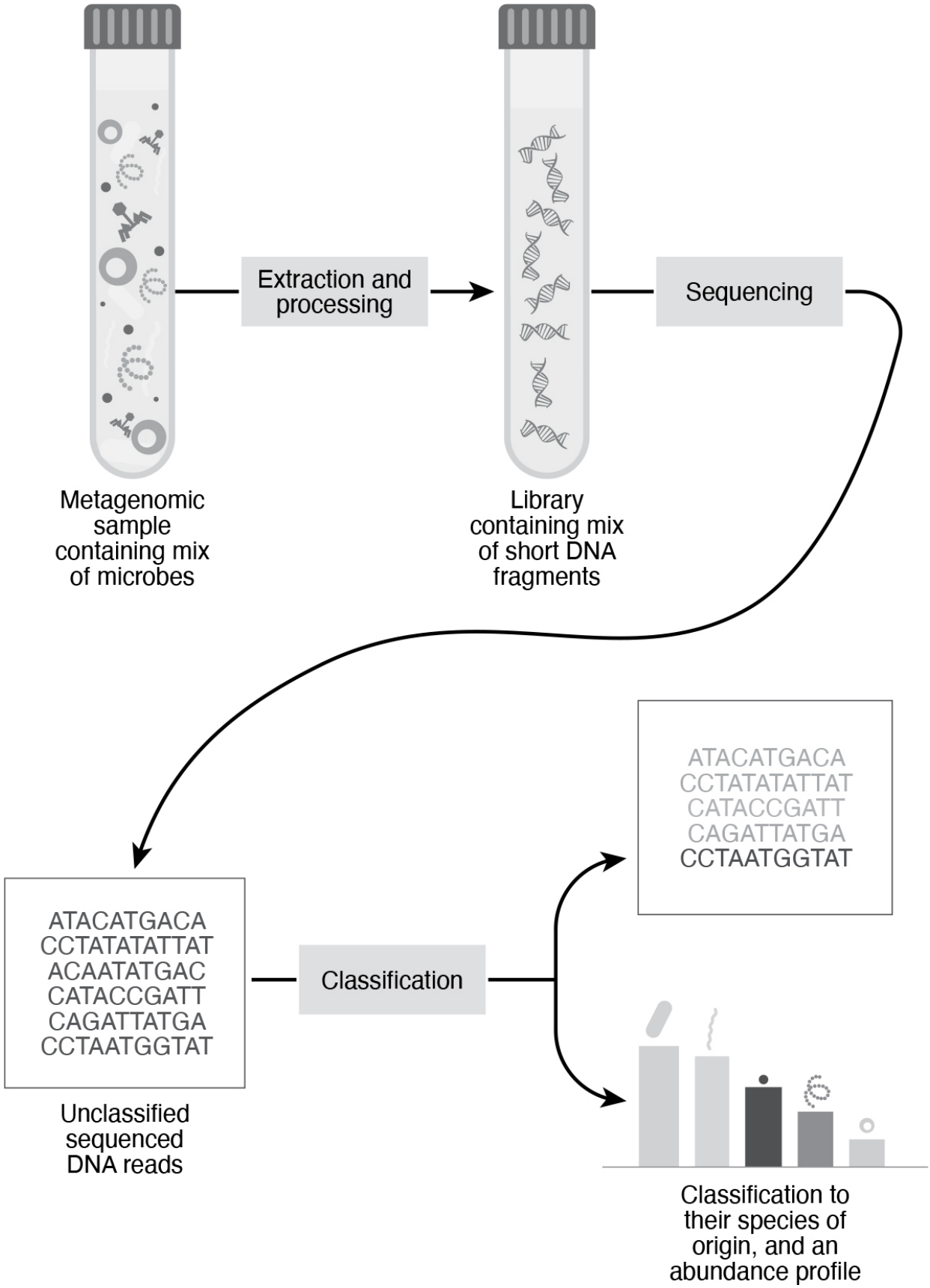

Extraction and processing
Sequencing
Metagenomic sample containing mix of microbes
Library containing mix of short DNA fragments
ATACATGACA
CCTATATATTAT
ACAATATGAC
CATACCGATT
CAGATTATGA
CCTAATGGTAT
Classification
ATACATGACA
CCTATATATTAT
CATACCGATT
CAGATTATGA
CCTAATGGTAT
Unclassified sequenced DNA reads
Classification to their species of origin, and an abundance profile

***Figure 10.4.*** How metagenomic sequencing determines a disease-causing pathogen in a patient with sepsis. Adapted from Simon Ye et al., "Benchmarking metagenomics tools for taxonomic classification," *Cell* 178, no. 4 (August 2019): 779–94, https://doi.org/10.1016/j.cell.2019.07.010. Description 51

I can't think of any reason why this technology hasn't been universally adopted in health systems. It's a potential lifesaving use of sequencing that is many years overdue to become a standard of care. We need to get over our ritualistic culture and move on.

## INFECTION AND AMERICAN LONGEVITY

The average lifespan in the United States in 1900 was forty-eight years, and a dominant reason it has improved to seventy-nine years now is because of our ability to prevent and treat infections. We're making profound progress in so many ways, and progress is speeding up: fast-tracking potent vaccines against most pathogens, some that we never had a vaccine for before; understanding infections that are a root cause for diseases, as achieved with HPV and perhaps MS in the future; and tackling antimicrobial resistance, with AI, structural biology, and phage therapy. And there are much better ways to deal with sepsis, using AI for prediction and rapid diagnosis, and metagenomics to get to the right treatment quickly. The reduction in deaths and morbidity from infectious disease should drop considerably in the years to come. Commitment to promoting equitable global access for these advances can make such a difference to human health span, yet another lesson we should learn from the COVID pandemic.

Unfortunately, however, we are our own worst enemy. During the pandemic, anti-science and anti-vaccine movements were organized and funded; this has led to far fewer people deriving the net benefit of COVID shots, which has also extended to marked reduction of vaccination in children. As a result, we've seen the recrudescence of poliovirus and measles. On the one hand, we're making phenomenal advances in the science; on the other hand, these are being aggressively countered. To make progress in defeating infectious diseases, it will be vital to develop effective strategies to prebunk and debunk anti-science efforts. That social challenge to our political culture may prove to be our greatest challenge in continuing to add to life expectancy.

# 11

# Promoting Mental Health

The complex interactions between our mental and physical health have never been fully broken down, and likely never will be, but there's no question that they are interdependent. It is hard to see how we could extend health span without attention to both components. Just consider that, in experimental models and people, chronic stress has been shown to potentiate cancer and its spread. Or the evidence for a cause-and-effect relationship of mental health and healthy longevity. That's why, from a population view, it's more than sobering to see the current burden of mental health illness, affecting 21 percent, more than fifty million Americans.

One in four of those millions of Americans are unable to get the mental health care they need. That's in part because there is only one mental health provider for every 350 individuals. This shortage of mental health care professionals has been steadily worsening over many years, accelerating during and since the COVID pandemic. In the 2024 World Happiness Report, the United States rank fell to twenty-third place, a drop from fifteenth the prior year. (The top four rankings were all countries in Scandinavia.) What will it take to get people less anxious, stressed, and depressed, and more upbeat and happier, with relatively few trained professionals to help?

We are going to get to the more technological initiatives we can take, but let's first consider some things that aren't talked about much in the medical

profession.

We don't spend nearly enough time outdoors in natural environments. The diverse health benefits that have been reported include lower blood pressure, improved sleep health, reduced risk of cardiovascular disease, improved cognitive function, better immune system response, reduced stress (with lower cortisol levels), and alleviation of symptoms of depression. A key mechanism that has been raised for the benefit of time in nature, and promotion of well-being, is activation of the olfactory pathway. Among nearly twenty thousand nationally representative participants, good health or high well-being was significantly greater with more than 120 minutes of exposure per week to nature, such as parks, woodlands, or beaches (fig. 11.1).

A much larger study from over four hundred thousand UK Biobank participants with twelve years of follow-up showed that long-term exposure to residential greenness was associated with a significant reduction of depression and anxiety (15 percent from highest to lowest quartile). Further analysis indicated that air pollution played a major role in this association. Less urban green exposure was assessed with functional MRI, identifying a neural risk factor of activation in the prefrontal cortex with impaired human emotional processing. Andreas Meyer-Lindenberg and colleagues at the University of Heidelberg concluded: "Our results validate the causal influence of urban green space exposure as a protective factor for human well-being in real life."

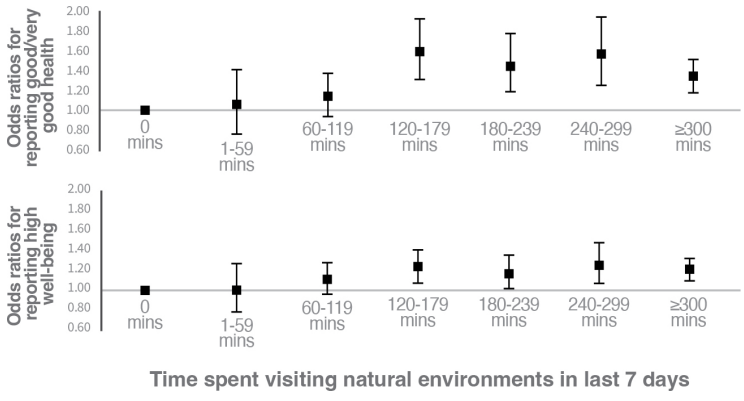



***Figure 11.1.*** Well-being and time in nature. Adapted from "Loneliness and social isolation linked to serious health conditions," Centers for Disease Control and Prevention, April 29, 2021, https://www.cdc.gov/aging/publications/features/lonely-older-adults.html. Description 52

That's just scratching the surface for the body of evidence. An exhaustive review of green space exposure in more than 140 studies emphasized the benefit for reducing all-cause mortality and fewer physical and mental ailments. Now a NatureScore has been derived from aggregation of satellite images, air and noise pollution, open water, and trees to provide a high-resolution map across the United States, accessible through a smartphone app. There is marked variability in the score across the country, ranging from approaching one hundred in Suffolk, Virginia, to fewer than ten in Union City, New Jersey. You can get your local score here: https://www.naturequant.com/naturescore/. From the map, it is also clear that accessibility to nature is substantially reduced where people of low socioeconomic status live, exacerbating health inequities.

A study based on the NatureScore in Texas using 61 million outpatient encounters, of which about 370,000 visits were for mental health, found a correlation of higher NatureScore and lower mental health utilization across 1,169 zip codes. Significantly less stress anxiety and depression were seen in places with a NatureScore above eighty, adjusted for socioeconomic and demographic factors. Residential green space was assessed in the Nurses' Health Study cohort of more than thirteen thousand middle-aged women, with better scores on cognitive tests, psychomotor speed, and attention associated with quantity of ground vegetation. Besides the link with mental health, a meta-analysis of all green space impact on health studies provides evidence for its association with reduced cardiovascular mortality and both the prevalence and incidence of diabetes.

Acknowledging the nature deficit disorder, nature "prescriptions" from health or social professionals are coming into vogue, recommending a fixed amount of time per week in a park or other natural setting. A comprehensive review of all randomized and observational studies of nature prescriptions showed less anxiety, less depression, improved blood pressure data, and increased physical activity. After reviewing all these papers, I've begun prescribing nature walks and visits to my patients, an opportunity I have missed until now.

One thing you will hear in nature, and you don't have to travel into exotic wilderness, is birdsong. From nature's music to the myriad human versions, the health effects are more powerful that we tend to think.

The magic of music to help alleviate symptoms from a variety of illnesses and reduce anxiety, stress, and depression has led to a whole new field in medicine—music-based interventions. In 2017, the National Institutes of Health had its first Music and the Brain workshop; it reconvened hundreds of experts at the end of 2023 to review the progress made in the science of music and to plan the next phase of research, including randomized trials.

At a more recent Music as Medicine conference, among many new studies reported, one was a music-based intervention to reduce chronic pain. That turned out to be substantially more effective when patients sang in a group rather than individually. A small, randomized study of singing for thirty minutes showed improvement of vascular endothelial function in older adults with heart disease. While there's still so much to be learned about music's potential therapeutic effects, advances have been made in unraveling some of the impact on brain circuits using brain MRI, electroencephalograms, transcranial magnetic stimulation, behavior capturing technology, and experimental models such as the songbird. Breaking music down to its three major elements, melody, harmony, and rhythm, alongside mode of delivery, solo or group, passive (listening) or active (performing), helps to dissect the specific effect. The auditory pathway that first processes music appears to be linked to motor and affective neural networks. These connections show benefits for Parkinson's disease, stroke, anxiety, and stress. The interlink between auditory and cognitive or sensory system processing may underlie potential impact in Alzheimer's disease, pain, or cardiovascular diseases. This may also promote healthy interoception: how the brain senses the body, interpreting and integrating body signals. For the latter, body maps were created for hundreds of participants' perceptions of chord progressions, particularly those with surprise or uncertainty features. A consistent finding from brain imaging assessments is the activation of reward circuits, which aligns with the perception of music-induced pleasure. It's fair to say that it's still early in the music-based intervention field for proof of efficacy, but surely there is no mental health disadvantage to enjoying music, whatever your personal taste—Bach, Beatles, or Taylor Swift.

## YOUR BODY ASLEEP, IN ACTION, AND IN TOUCH

Let's consider some of the less surprising lifestyle+ factors that bear on our mental health.

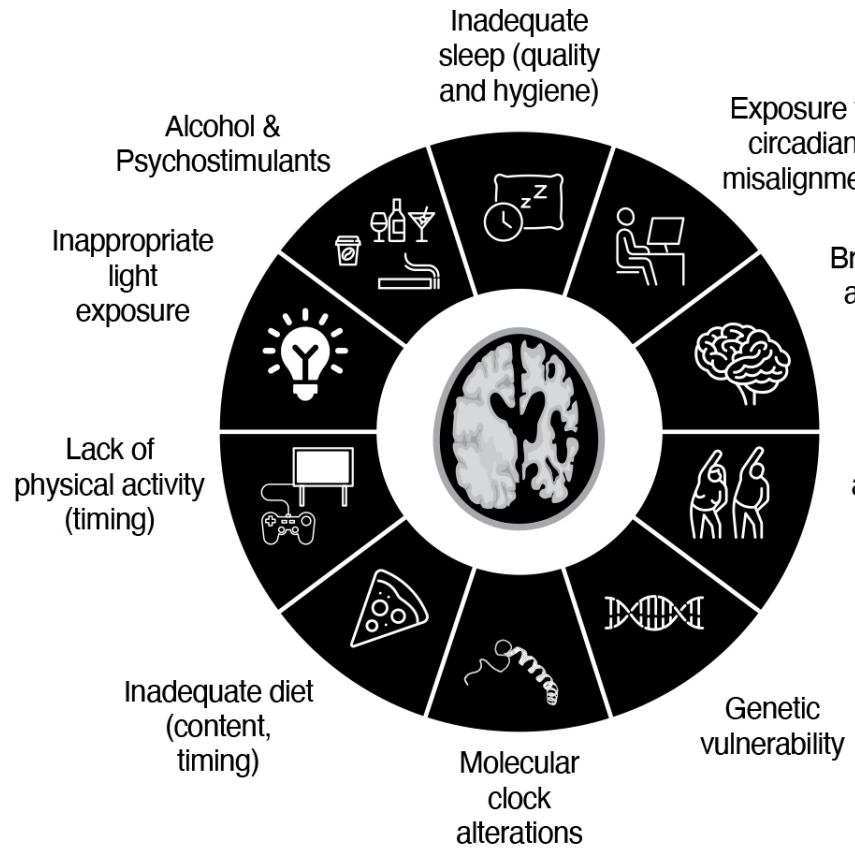
Many of us have noticed and research has confirmed that after a good night's sleep, exercise performance is enhanced, glucose regulation is improved, and mood is uplifted. Digging a little deeper, inflammation is reduced, partly mediated by the gut microbiome and the gut-brain crosstalk. Chronic sleep deficiency increases the risk of metabolic syndrome and obesity. Sleep and circadian rhythm are tightly interlinked, and recent work with continuous wearable sensors has demonstrated a causal relationship between sleep or circadian disturbance and bad moods.

Although I previously reviewed the role of sleep health for promoting cardiovascular, metabolic, and neurologic health in part 2, its interconnection with mental health is fundamental. A key mechanism is the clearance of metabolic waste products during sleep. Unfortunately, sleep disturbance increases in older adults and is a risk factor associated with late-onset dementia. That's one of many factors that affect sleep health, as shown in figure 11.2, which include lack of physical activity, alcohol and stimulants, poor diet, inappropriate light exposure, and genetic variation. In people with mental health conditions, objective sleep metric alterations are characteristic. There are many ways to improve sleep health, as the graphic (lower panel) summarizes: increasing physical activity, decreasing alcohol and caffeine, keeping on a regular schedule of bedtime and awakening, timing of eating, and optimizing exposure to natural daylight while blocking out all light during sleep. Avoidance of late-night screen time is another key component of optimal light management. Darkness is a prompt for the pineal gland to produce melatonin. There are limited data to support taking a melatonin supplement to get to sleep; more evidence exists for its help reducing jet lag. No supplements or medications have been shown to be effective and safe for chronic use, and particularly none have been demonstrated to promote metrics of high-quality sleep, such as the most restful, non–rapid eye movement kind. Sleep metrics can be accurately monitored with wearable sensors such as the Oura ring, which has been assessed in multiple small, randomized trials and

summarized by my team at Scripps Research. Even though such data correlates with formal sleep lab studies, it remains unproven that daily monitoring will durably improve sleep health. But sensors may be useful for a person to understand the specific factors that influence their sleep, like seeing the reduction of alcohol intake or late-night screen time having a positive impact.

There is a digital intervention to promote sleep quality that has been getting more intensive study in randomized trials and large real-world assessment: digital cognitive behavioral therapy (dCBT). Interactive dCBT can be accessed through mobile apps or computers and tailored to the individual, exploring the background factors, providing education, collecting data, reducing stimulation, and overall coaching for sleep pattern improvement. The American Academy of Sleep Medicine reviewed more than fifteen apps. Some are available free, like CBT-i Coach, while others require a prescription and are FDA cleared, such as Somyrst (available through Nox Health). Direct randomized comparison of the digital alternative to in-person psychotherapy CBT for improving sleep suggests the former may approximate the latter, but it is far more practical and much less expensive.

Continuing this discussion in the lifestyle+ dimension, we've long known about the relationship between physical activity and mental health, including consistent reduction of anxiety and depression, but that was all reinforced by the largest systematic review of over two hundred randomized trials of over fourteen thousand participants with depression. The results, as summarized below (fig. 11.3), showed significant improvement with walking and jogging, yoga, all forms of aerobic exercise (including dancing and cycling), strength training, tai chi, and qigong. Notably, the most common form of medical treatments—selective serotonin reuptake inhibitors (SSRI)—were barely effective, much weaker than exercise. CBT, as mentioned for promoting sleep health, had a similar magnitude of impact as many types of physical activity. The intensity of exercise was correlated with the extent of improvement, but low-intensity walking and yoga had meaningful benefit.


Inadequate sleep (quality and hygiene)
Exposure to circadian misalignment
Brain activity alterations
Changes across the lifespan
Genetic vulnerability
Molecular clock alterations
Inadequate diet (content, timing)
Lack of physical activity (timing)
Inappropriate light exposure
Alcohol & Psychostimulants

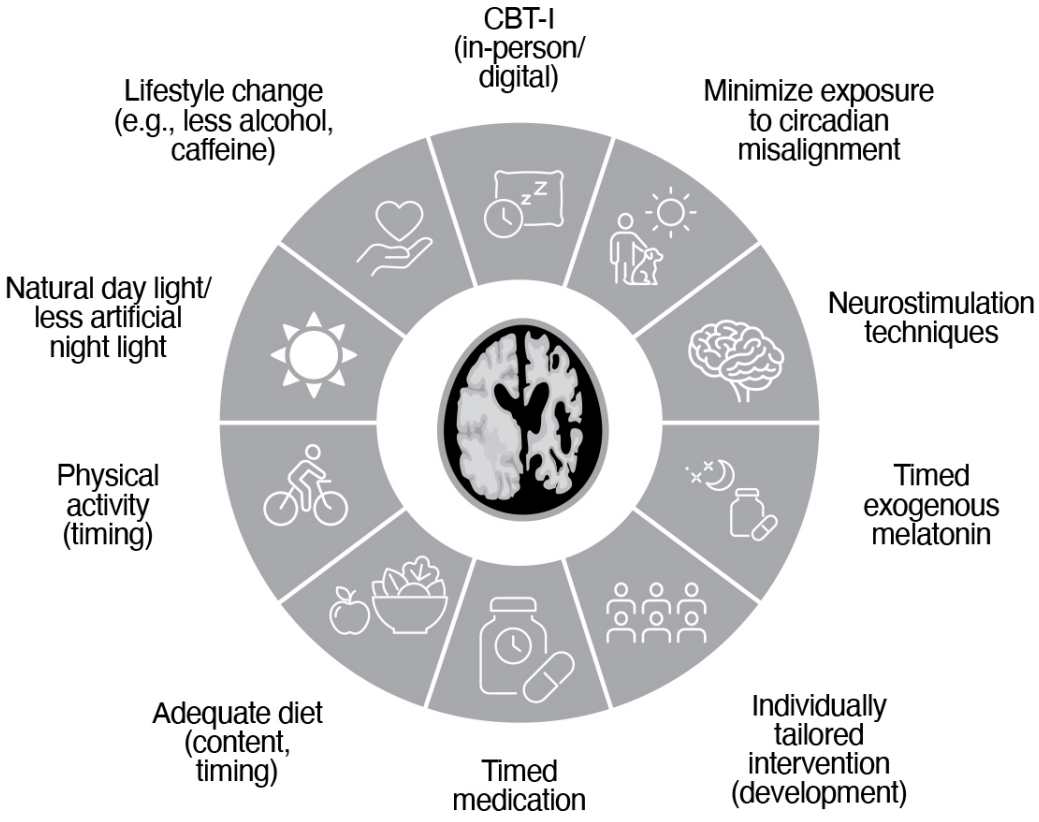

CBT-I (in-person/ digital)
Minimize exposure to circadian misalignment
Neurostimulation techniques
Timed exogenous melatonin
Individually tailored intervention (development)
Timed medication
Adequate diet (content, timing)
Physical activity (timing)
Natural day light/ less artificial night light
Lifestyle change (e.g., less alcohol, caffeine)

***Figure 11.2.*** Factors affecting sleep health (upper panel) and potential interventions (lower panel). Adapted from Nicholas Meyer et al., "The sleep–circadian interface: A window into mental disorders," *Proceedings of the National Academy of Sciences of the USA* 121, no. 9 (2024): e2214756121, https://doi.org/doi:10.1073/pnas.2214756121. Description 53

Those findings are further strengthened by a meta-analysis of forty-one randomized controlled trials of exercise for a major depressive disorder that markedly reduced symptoms of depression. Even light exercise such as gardening led to improvement, and, independent of type of physical activity, one of every two individuals experienced reduced symptoms, somewhat more than what is seen with medications or psychotherapy.

In a study of the mental health benefits of exercise with more than fifty thousand participants, including a subset that underwent brain imaging, physical activity reduced stress-related neural activity in the amygdala and reduced cardiovascular risk. Participants with and without depression benefited, but the ten-year cardiovascular risk showed a more striking reduction in people with depression. Besides improvement in mood and reduction of cardiovascular risk that occurs with regular exercise, a recent study indicates that physical activity mitigates social isolation and loneliness.

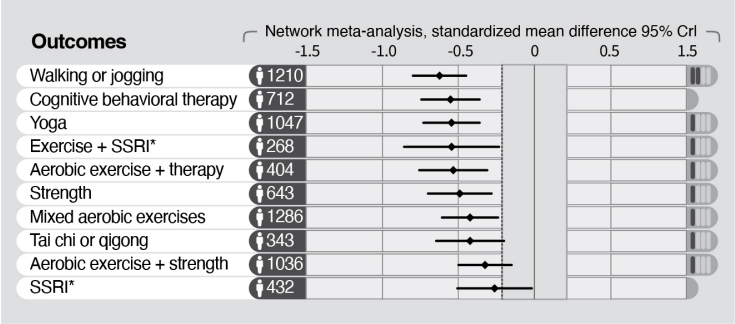



***Figure 11.3.*** Different types of physical activity and SSRI medications for reduction of depression. The horizontal lines to the left of zero indicate a significant effect; SSRI drugs crossed the line of zero, which means their effect was not significant in this systematic review. Number of people for each activity type or SSRI intake shown in second column. Adapted from Michael Noetel et al., "Effect of exercise for depression: Systematic review and network meta-analysis of randomised controlled trials," *British Medical Journal* 384 (February 2024): e075847, https://doi.org/10.1136/bmj-2023-075847. Description 54

Although related, loneliness and social isolation are subjectively different. Loneliness, a state of mind, refers to feeling alone and dissatisfied with one's social relationships; social isolation simply describes a paucity of social contacts.

Contributing factors to the perception of loneliness are losses and transitions, but they are generally heterogeneous and individualized. Both loneliness and social isolation have been on a steep rise for decades but defy the need, embedded in our DNA, to connect with people. As we age, our time spent alone increases (fig. 11.4). Also, the proportion of one-person households around the world has markedly increased and is well above 60 percent in many American cities.

My friend Vivek Murthy, a former surgeon general of the United States, wrote the important and inspirational book *Together: The Healing Power of Human Connection in a Sometimes Lonely World*, which summarized the evidence. Loneliness, a sign of poor social health, is linked to increased risk of depression, anxiety, and suicide. Survivors of cancer who reported loneliness had a higher mortality risk during four-year follow-up compared with those with no or minimal loneliness. Social isolation is associated with a significant risk of premature death of similar magnitude to that of smoking, obesity, and physical inactivity as well as a 50 percent increased risk of dementia. Among over 430,000 UK Biobank participants with a mean age of fifty-seven years and followed for nearly fourteen years, loneliness and social isolation were both associated with a significant increase of lung cancer. This study incorporated genetic evidence (Mendelian randomization) that also showed that not living alone was linked to about an 80 percent reduced risk of lung cancer.

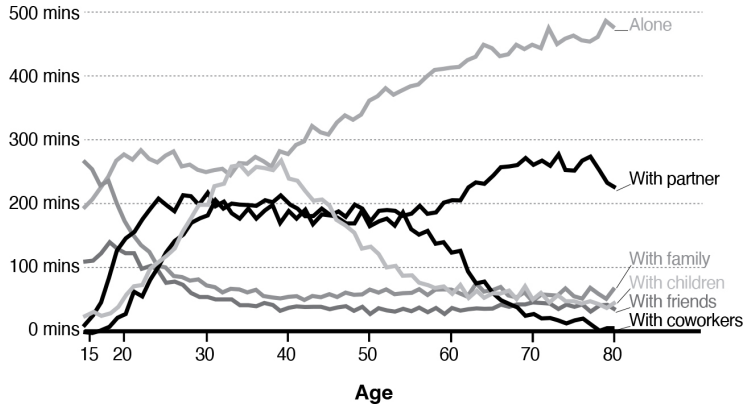



***Figure 11.4.*** How Americans spend time by age groups. Adapted from "Who Americans spend their time with, by age," Our World in Data, accessed September 5, 2024, https://ourworldindata.org/grapher/time-spent-with-relationships-by-age-us. Description 55

You will remember Mrs. L. R., age ninety-eight, from the introduction. At age ninety-four, when her husband died, she was devastated, lonely, and depressed. One of her seven women friends with whom she has played cards and Rummikub every week for decades suggested she relocate to a senior residence. Within a day of moving into that facility, another resident artist contacted her and set up a dinner for her to meet the other artists in the building. A new network of senior women artists evolved, enriching Mrs. L. R.'s social connections, so much so that she now must strike a balance between the solitude she enjoys for painting and doing jigsaw puzzles and the time she plays cards or interacts with her new artist group.

That exemplifies how other people took initiative to help remedy loneliness and isolation that she was unable to do on her own, leveraging her past contacts, her hobby, and her willingness to relocate to a supportive community. A delightfully vibrant and energized near-centenarian, you wouldn't think she previously suffered from marked loneliness and isolation.

An aspect of our lives we tend not to talk about much is human touch. A systematic review of being touched by other people in both adults and newborns highlighted its critical importance for mental and physical health. From 137 studies in approximately thirteen thousand individuals, skin-to-skin touch significantly improved sleep, blood pressure, and mobility, while reducing fatigue, anxiety, pain, and depression. Whether it's a one-off brief hug or handshake or a sixty-minute massage session, human touch appears to be consistently therapeutic, perhaps to an even greater extent among women.

## FOOD, MOOD, AND STRESS

When a fecal transplant from a person who is depressed is given to rodents, the animals exhibit behavioral features characteristic of depression. Fecal transplants from young to old mice improve their cognitive performance and memory. We know from imaging the brains of the older mice after these transplants that age-associated inflammation (inflammaging) is reduced and the hippocampus region appears rejuvenated. In healthy human adults, a small randomized, controlled trial of high fiber and reduced processed food for one month lowered perception

of stress. The more adherence to that diet, the greater reduction of stress perception score.

The Brunner glands in the small intestine promote the proliferation of *Lactobacilli* bacteria, which are protective, enhancing immunity. Through vagal nerves, there is a direct connection between these glands and the brain's amygdala. With chronic stress, the neural circuit between Brunner glands and the brain is inhibited, suppressing the immune response.

Many other observational diet studies have reinforced the relationship between food and mood. The Mediterranean diet and other healthy diets with a low inflammatory index have been associated with less depression and improved mental health compared with unhealthy Western diets. The Mediterranean diet in six large studies showed more than a 30 percent reduction of depression, which was similar to the magnitude of reduction for other healthy diets. In contrast, in two cohort studies, among more than eighty-five thousand participants, a diet with high glycemic index of refined carbohydrates and sugars has been linked to a higher risk of depression. Further, repeated episodes of hypoglycemia have been linked to mood disorders. A systematic review of thirteen studies of vegetarian and vegan diets suggested a mixed effect: they were associated with an increased risk of depression and lower anxiety scores.

There are, of course, many potential confounding factors for evaluating these cohort and cross-sectional studies since the relationship between food and mood is bidirectional. But generally, the theme of avoiding poor diets that induce systemic inflammation, like those high in ultra-processed foods, and favoring diets with a low inflammatory index correlated with better mental health. Once again, the gut microbiome is central in modulating our immune response, so there is an interdependence between stress, immunity, and the gut microbiome. It's been referred to as the "holy triad" underlying depression.

The brain-gut microbiome axis is likely playing a bigger role than anticipated for our response to stress. From combined analysis of multiomics of fecal samples (DNA, RNA, metabolomics) and brain MRIs in over one hundred participants, certain bacterial species and their metabolites were correlated with resilience to stress or vulnerability to depression and anxiety symptoms. Whether pre- or

probiotics can promote resilience to stress needs to be assessed, but, if confirmed, this will further highlight the gut microbiome's impact on our mental health.

Given what we have learned about the GLP-1 family of drugs for obesity, we have to wonder what influence they may have on mental health. All the data reviewed by regulatory agencies thus far do not support this drug class leading in any way to depression or suicide. On the other hand, this family of drugs' ability to suppress systemic and brain inflammation, a well-recognized feature of depression, along with improvement in self-image after substantial weight loss, may ultimately prove to be important for reducing depression and anxiety.

Obviously, we need drugs that can do that. The mental health toll of the COVID pandemic and climate change have taken the burden of stress, anxiety, and depression to new heights. A global survey for eco-anxiety found that 60 percent of people are extremely or very worried about climate change. There's no shortage of speculation about secondary ill-effects increasing vulnerability to diseases. For example, does anxiety or depression increase the risk of cancer? While this has been generally accepted as true, an important analysis of all the data available across eighteen cohorts, involving more than three hundred thousand participants, indicated it's not the case, apart from lung and smoking-related cancers. But it's not so clear-cut. Although chronic stress activates the immune system, it appears to do so in a way that disrupts the ability of our immune cells to fight cancer and may impede cancer treatments. "Good stress" is adaptive and helps build resilience, ability to cope, and neuroplasticity. In contrast, "bad stress" is maladaptive, which is a way of saying that it has a negative biologic consequence. Chronic bad stress is a trigger for mood and anxiety disorders, which increases vulnerability for diabetes and cardiovascular disease. In a study of more than 211,000 people in Sweden with over twenty years of follow-up, nearly 8 percent developed depression, anxiety, and stress-related disorders. These individuals had significantly higher blood glucose levels and triglycerides as well as lower HDL values, raising the possibility that metabolic dysregulation is associated with risk across the spectrum of these mental health conditions. But how do we know if our response to stress is healthy ("good") or bad? Maybe there is a new technological answer.

## FROM DIGITAL TRACKING TO CHATBOTS

Depression, anxiety, and stress-related disorders have traditionally been diagnosed and monitored subjectively, through self-report and interviews. Wearable sensor technology has been making significant inroads to provide objective metrics such as activity and sleep patterns, frequency and duration of phone and text interactions, light exposure, heart rate and heart rate variability, galvanic skin response (electrodermal activity), smartphone facial expression mood capture, emotions via voice tone and inflection, and sweat biomarkers.

This recent move toward embracing digital technology is dramatic. More than 55 percent of all mental health visits with clinical practitioners are done virtually.

Although sensor capture of continuous or high frequency data from individuals in their real world is now feasible for tracking mental health, it has yet to be used in clinical practice at any scale. There are challenges with data analytics, adherence, privacy and security of data, and lack of compelling evidence from randomized trials in diverse populations.

Mindstrong was one of the first companies to pursue digital tracking for mental health, using a person's interaction with their keyboard for typing speed, typos, and tapping and scrolling patterns to reflect changes in mood. In the rush to market, this digital biomarker was never adequately validated in clinical trials, and there was lack of trust and engagement from clinicians, ultimately leading to the demise of the company in 2023. Perhaps the failure will be instructive for the many other companies who are working on digital tracking for mental health.

The results from 145 randomized trials of mobile phone apps for mental health with nearly forty-eight thousand participants did not provide any strong evidence of benefit, although some of the studies for anxiety, stress, and depression were graded as "providing highly suggestive evidence" of slight effects. Meditation apps like Headspace and Calm, which use soothing audio and breathing exercises, have become popular, but the evidence for reduction of anxiety and stress remains underwhelming. On the other hand, a large randomized controlled trial of self-administered mindfulness interventions, including those directed to breathing and walking, did reduce short-term stress.

The prospects for dCBT apps are somewhat more favorable. A recent review of fifty-six randomized trials found about a 25 percent reduction of depression and anxiety metrics, especially notable in Internet-based dCBT trials with larger effect sizes and longer duration of benefit. The smartphone app Rejoyn, by Otsuka Pharmaceuticals, for people with at least moderate depression, was the first to be FDA cleared for mental health, even though it did not fulfill the clinical trial's primary endpoint. Used as an adjunct to antidepressant medication treatment, in a randomized trial of 386 participants who received the app compared with sham controls, there was a modest improvement in a depression rating scale. In a randomized trial of a therapist-supported dCBT for reducing anxiety and depression in 194 people with chronic physical conditions, twelve weeks of therapy led to a significant reduction of psychological distress during the COVID-19 pandemic. The benefit was more substantial than all previous trials and in comparison to eighty-three digital interventions for depression in over fifteen thousand participants. Similarly, dCBT significantly reduced postnatal depression and anxiety in a randomized trial of twelve hundred pregnant women. There is no shortage of non-FDA-cleared dCBT apps on the market, including Sanvello, MindDoc, Happify, Evolve, CBT-i Coach, Mindshift CBT, MoodMission, and many others, most of which charge, with a range from $5 to $50 per month. Many of these are indexed at mindapps.org.

Flotation therapy is getting popular in the United States, with an estimated four hundred float centers at which people can float in a tank of warm salt water for about an hour. The limited evidence for helping mental and physical health is based on two randomized trials that provided modest data supporting a salutary effect on sleep, blood pressure, muscle tension, and anxiety symptoms.

In contrast to passive floating, another emerging nonpharmacologic intervention for anxiety, stress, and depression is neurostimulation. This treatment involves either noninvasive transcranial magnetic stimulation or transcranial direct current stimulation, which activates cortical neurons via the scalp. It has been used in a range of mental health disorders. A review of over two hundred randomized controlled trials revealed evidence of benefit for generalized anxiety disorder and for depression. These stimulation techniques involve twenty to thirty sessions over four to six weeks, but there are refinements to reduce that

course of therapy for transcranial magnetic stimulation by using fMRI guidance and intermittent theta burst stimulation. That bespeaks the issue of marked variability in results for different regions activated that may benefit from better individualized approaches. Since the mechanism for brain activation is still poorly understood, and mapping brain circuitry at the individual level is in early stages, neurostimulation to help promote mental health can be viewed as promising with much work and validation that needs to be done. Demystifying brain circuitry will help, as exemplified by electroconvulsive therapy for severe depression, introduced almost a century ago, and we still do not understand how it works—or doesn't.

Let's turn to a less violent electrically engineered therapy. The use of chatbots to help relieve anxiety, stress, and depression goes back several years before ChatGPT and the new era of generative AI became the rage in late 2022. They deliver dCBT principles but have added interactivity, simulating physical CBT, but providing 24/7 accessibility and scalability. Now about one-third of employers provide a digital therapeutic for mental health support, many of which are of this type.

Alison Darcy is a psychologist who founded Woebot Health, one of the early mental health chatbots that conducted multiple small randomized trials showing they reduced anxiety and depression. She and Woebot's chatbot, which uses rules-based, unimodal AI and has garnered more than 1.5 million users though employer benefit plans, were featured on *60 Minutes*. That segment questioned the safety of generative AI for supporting mental health as compared with the simpler, older unimodal AI, and for less chance of providing harmful advice.

The insightful essay "My Year with Woebot" in the *New York Times* began as follows:

> I was initially skeptical of Woebot. The idea seemed almost too simple: an app on my phone that I could open when I needed it, type my hopes, fears and feelings into, and, in turn, receive AI-generated responses that would help me manage my emotions. There are plenty of good critiques of apps that claim they can provide therapy without the therapist: How could an algorithm ever replace the human touch of in-person care? Is another digital intervention really the solution when we're already so glued to our phones? How comfortable was I being vulnerable with an app that could track my data? Spending time with Woebot didn't really bring me answers to these important questions. But I did discover that, despite them, I'd become weirdly attached to my robot helper.

The writer, Barclay Bram, ended up deriving marked relief of anxiety and depression. He was thankful for the chipper little bot checking on him, and appreciated Woebot the more he used it. Of course, that's just an anecdote. What is the research on mental health AI chatbots telling us?

A systematic review of fifteen randomized trials of AI chatbots for mental health has shown a significant reduction of depression and psychological distress that was more pronounced than with multimodal, generative AI. There was no improvement noted for overall psychological well-being. Another review of ten studies, seven of which were randomized trials, conducted in women, concluded similarly that chatbots were effective in reducing anxiety, distress, and depression. In a related review of nineteen trials for chatbot impact on lifestyle, physical activity, healthy diet, and sleep health were promoted.

Results of an important study conducted in England with the Limbic AI chatbot in nearly 130,000 participants countered the belief that this technology would worsen inequalities. Self-referrals for mental health through Limbic were substantially increased among minority ethnic groups (29 percent increase) and nonbinary individuals (179 percent increase), "providing strong evidence that digital tools may help overcome the pervasive inequality in mental healthcare."

While these findings with conversation chatbots and dCBT are encouraging, the concern about privacy cannot be emphasized enough. It is worth reviewing the privacy policy, security measures, and whether the companies sell information to third parties. This is also an issue that requires more regulatory oversight. Adopting policies that prevent breaches, sale, or any compromise of an individual's data or engagement is essential.

The need for oversight extends to safety, with notable precedents, such as a chatbot that was designed to help with eating disorders but instead provided harmful advice to make matters worse, necessitating its removal by the National Eating Disorders Association.

What about the next generation of chatbots that use generative AI? Large language models have been assessed for their ability to track people's mental states, and they compared favorably to human performance for a comprehensive battery of tests. Generative AI, integrated with therapeutic sessions, has specific potential to improve behavioral health by recognizing, expressing, and managing

emotions. However, the important risks of harm from generative AI wellness apps, which do not go through regulatory oversight and can be used in unintended ways, have been emphasized. It's still too early to determine the net benefit of generative AI for promoting mental health, but there's considerable optimism, especially for reduction of anxiety. Not as a substitute for a therapist but as an adjunctive means of support.

## LAST RESORTS

So the problem remains. With relatively fewer trained professionals to provide mental health care, how will we help older people feel less anxious, stressed, and depressed? Medications should be considered as the absolute last resort. Again, many lifestyle factors work as well or better than medications. That's for the common mental health issues, not serious ones like bipolar or major depression disorder, schizophrenia, or post-traumatic stress disorder, which are beyond the scope of our principal focus of expanding health span.

However, the use of selective serotonin reuptake inhibitors is remarkably common, with approximately one in eight Americans taking one of these drugs (the common ones are Prozac, Zoloft, Celexa, Paxil, and Lexapro). The "serotonin hypothesis," which dates to the 1960s, is controversial and has been challenged, but evidence remains that depression is a disorder of serotonin-based signaling in certain regions of the brain. More recently, we've seen the broadening of medications for depression from the classic SSRIs to potentially include ketamine and psychedelics. The problem with all these drugs—SSRIs, ketamine, and psychedelics—is that we don't know they work clinically even though, for example, ketamine is known to increase extracellular serotonin levels. Oral ketamine tablets, assessed in a randomized trial for treatment-resistant depression, demonstrated some efficacy. A systematic review of nine randomized trials of psilocybin, a psychedelic, indicated self-reported improvement of symptoms of depression. A single dose of psilocybin in a small study of healthy adults undergoing serial functional MRI showed a massive dissolution of brain circuitry networks, particularly those involved with perception of space, time, and self, some of which persisted for weeks.

We have much to learn why medications don't work at all for many people. Then there's the long list of side effects. With improved understanding of brain circuits and inflammation, there's a renaissance in new drugs for mental illness and an optimistic outlook for the emergence of "precision psychiatry." Blood biomarkers of inflammation have been linked to lack of response to SSRIs and ketamine. The use of AI to predict the optimal treatment for depression for an individual is being actively pursued. Structure-based drug design and discovery have given rise to multiple new drug candidates that are entering clinical trials. It's early, but there's clearly good reason for hope that we'll see better understood, more effective, and fewer side effect pharmacologic approaches in the future.

We can't meaningfully promote healthy aging without attention to its interdependence on our mental health. We all experience stress, but it's good and adaptive if we use it to exercise and double down on key lifestyle+ factors that are known to help. That includes nature, music, and social interactions. With the ongoing digital transformation of mental health support, a conversational chatbot or digital CBT app is just a click or download away. Ironically, at a time when there is the greatest mismatch of need for mental health support and lack of available professionals, there are more ways to get immediate 24/7 access for nonhuman help than ever before. As large language models continue to improve, the latter will undoubtedly get further validation, help reduce inequities (as we've already begun to see), and become more alluring with more use of human avatars than just text or voice. It is hard to imagine how human touch could be fabricated, but who knows? In any case, we will always need real human touch, metaphorically and literally. But anything, including an engaging AI chatbot or avatar that truly promotes our mental health, is something not to miss in our quest to age healthily.

# PART IV

## Thinking Ahead

# 12

# Changing Aging

Have you visited the longevity lifespan circus lately? There are eight hundred longevity clinics in the United States—even at the prestigious Mayo Clinic—so they are hard to miss. Some charge up to $50,000 per week. There are longevity vacations with intravenous drips; "Rejuvenation Olympics"; a Fountain Life "country club for precision diagnostics"; fitness centers with intravenous antiaging drips; an X-Prize awarding over $100 million to restore at least a decade's worth of muscle, brain, and immune function in older adults; and stem cell clinics for longevity. There are carnival barker scientists making false claims and hawking supplements as well as longevity leader lists for what dose of rapamycin they are taking to extend their lifespan. There's an outspoken forty-seven-year-old centimillionaire taking over 110 supplement pills a day, monitoring his organs (which includes a penile-ring erection sensor) with a staff of thirty medical specialists, spending more than $2 million for food each year for a precise daily intake of 1,977 calories, who has received plasma infusions from his teenage son, along with gene therapy.

Then there are the many hundreds of companies developing drugs or interventions to promote lifespan or selling biomarkers to track one's biological age. Many high-profile billionaires, such as Jeff Bezos, Sam Altman, Yuri Milner, and Peter Thiel, are betting big on some of these companies. I don't claim to have any special access to the motivations of these successful businesspeople, but I am

reminded of a line from Venki Ramakrishnan in *Why We Die*: "When they were young they wanted to be rich, and now that they're rich they want to be young." These entrepreneurial investments aren't likely to settle down anytime soon because we're seeing some real progress in understanding the science of the aging process. Unfortunately, that has led to irrational exuberance in many dimensions. In this chapter, I'm hoping to set the record straight on what we know, what we don't know, and how likely it is we'll be able to modulate the aging process to some extent one day. Not yet.

It is true that we are in the midst of changing our perception of what "old age" means. For a start, there are simply more centenarians around. There were 120,000 people aged one hundred and older in the United States in 2024, and that number is expected to rise to about 600,000 by 2060. Unlike our healthy aged exemplar, Mrs. L. R., there's little evidence we've conquered frailty—we seem to be as vulnerable to age-associated decline as ever. In fact, more so. The result of more people getting older with multiple chronic conditions.

According to the World Health Organization, the average American celebrates just one healthy birthday after the age of sixty-five. That seems bleak, but another way to express this acceleration of morbidity with older age is by the number of chronic conditions. For Americans aged sixty years and above, 95 percent have at least one chronic disease, such as heart disease or diabetes, and 79 percent have at least two chronic conditions. Life expectancy in the United States is starting to come back with a small increase after the COVID pandemic but is still below its peak a decade ago, and well below many peer countries in western Europe and Asia.

Life expectancy (LE) and health-adjusted life expectancy (known as HALE) are quite different. LE is the average number of years that a person at a given age is expected to live. In contrast, HALE is the average number of years spent in good health, and there's a wide spectrum of conditions that are considered to detract from "good health."

Over time, the gap between LE and HALE is getting larger, with chronic illnesses on the rise in the United States. That's why the notion that slowing down aging for one year, a small increase in LE, would be worth $38 trillion, and by ten years $367 trillion in the United States is not far-fetched. If health span is

not increased and the gap closed, we'd be adding to the economic burden of long lives with chronic diseases, not reducing it. The only way we might accomplish closing the gap is to slow the aging process, ideally at the whole-body level. If we reduce heart disease but increase the prevalence of Alzheimer's disease, we're into competing interests and a Whac-A-Mole model of expanding health span. A longer lifespan gives more chance for all the diseases of biological aging to manifest. People just don't fall off a cliff healthy. Slowing the whole-body aging process is the only way to bridge the gap.

There's a fierce debate about human lifespan's upper limit. Some researchers invoke the Gompertz-Makeham law, which states that the risk of death increases exponentially with age; others invoke the Hayflick limit theory, which posits that human cells cannot divide much beyond fifty times. The age at death of the world's oldest person—Jeanne Calment at age 122—hasn't budged since 1997. Beyond biology, there's the physics of wear and tear, cumulative aging from recurrent stress to our cells, tissues, and body, the obligatory decay that can be loosely attributed to the second law of thermodynamics. In this regard, whether there is an absolute limit is moot, since having more Methuselahs isn't going to promote healthy Methuselahs. Ideally, we'd want to extend both with no gap, achieving full alignment of HALE and LE. That's not so simple and will ultimately depend on exploiting the science of aging.

## THE BIOLOGY OF THE AGING PROCESS

The process of aging is generally considered to be the accumulation of damage to our cells and molecules over time. Biological hallmarks of the process indicate they occur with aging, their increased prominence accelerates aging, and getting rid of the hallmarks slows aging. The most cited paper on the hallmarks of aging was published in 2013 and updated in 2023. Over the course of that decade, there's been remarkable progress in the field only touched on by the expansion of hallmarks from nine to twelve (fig. 12.1), with the recent additions of chronic inflammation, the gut microbiome, and waste removal. This breakdown of twelve different processes is a bit arbitrary and reductionist because there is considerable overlap between them. They're *all* integrative, even though that's

only one of the three categories (fig. 12.1). However, each hallmark provides "the opportunity to decelerate, halt, or reverse aging by therapeutic interventions." Indeed, each process is being pursued by multiple companies and interventions. Let's go clockwise around the hallmark wheel, starting at genomics.

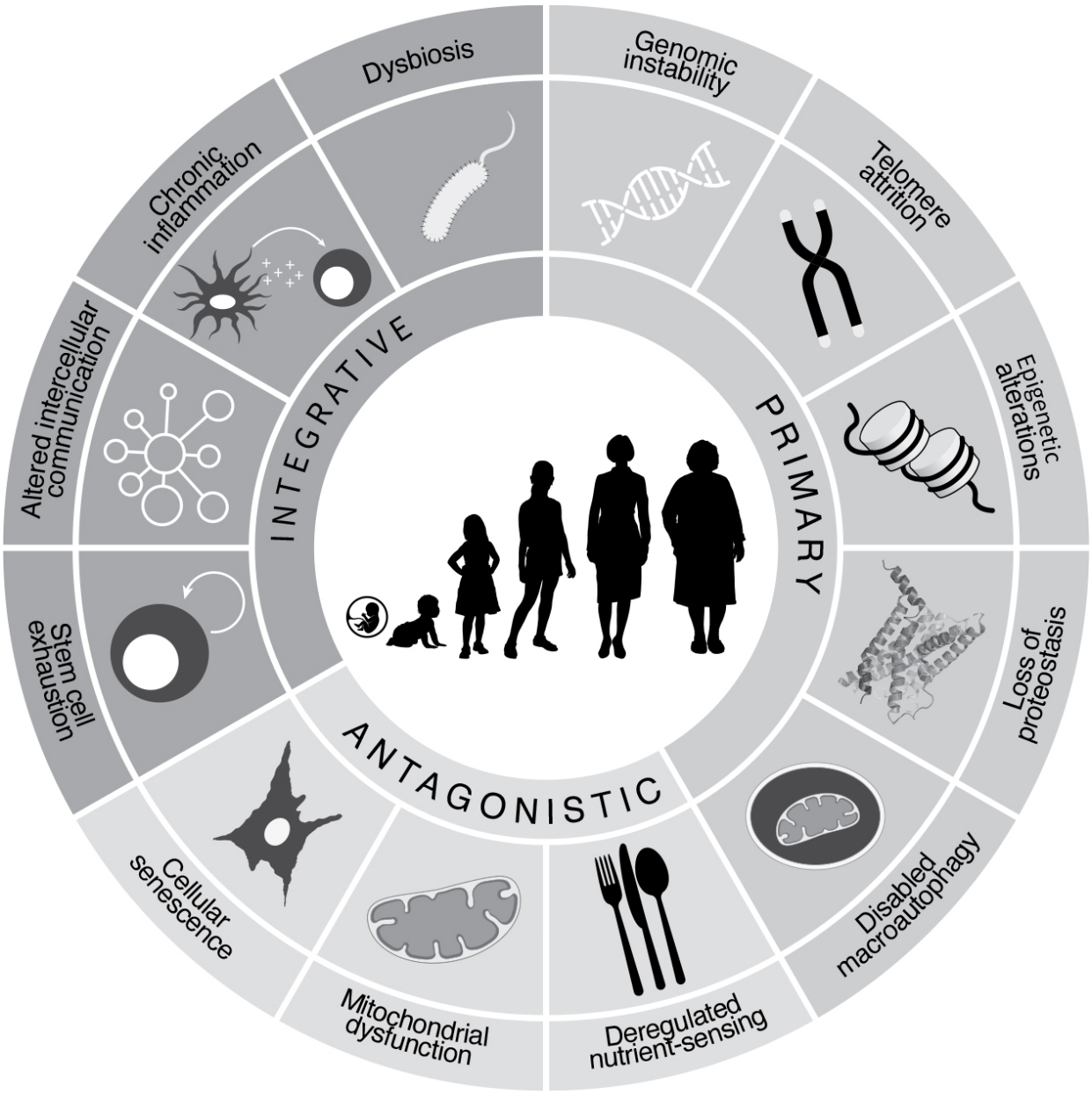



***Figure 12.1.*** The hallmarks of aging. Adapted from Carlos Lopez-Otin et al., "Hallmarks of aging: An expanding universe," *Cell* 186, no. 2 (January 2023): 243–78, https://doi.org/10.1016/j.cell.2022.11.001. Description 56

## Genome Instability

From the genome variants that are associated with longevity we can learn what genes and pathways are implicated in the aging process. That's especially true when it comes to rare variants present in less than 1 percent of the population, and with a high odds ratio or magnitude of effect, that track with human aging. They are found by genome sequencing, either by the exome (the roughly twenty thousand protein coding genes) or the whole genome of three billion letters. Common variants, occurring in more than 1 percent of the population, can be detected through genome chips (known as arrays). These chips have identified more than a hundred letter changes (single nucleotide variants) associated with lifespan, which can be aggregated to provide a polygenic risk score. Combining all the rare and common variants linked to longevity gives us a sense of which traits can be passed from parent to offspring. The estimates for heritability of longevity vary considerably: 12 percent in one of the most rigorous assessments in thirteen million people and well below that in others. That context is important, telling us that genetics is important in the inherited trait of aging, but its contribution is limited.

On the other hand, variations in genes consistently identified through sequencing studies provide insights into the underpinning of human aging. The same genes (with their principal function in parentheses) keep popping up from studies of people with extreme longevity or populations studies: APOE (lipid metabolism); FOXO3 (nutrient sensing); IGF-1R (insulin growth factor-1 receptor); CETP (lipid metabolism); CDKN2A/2B (cell cycle and senescence); certain HLA types (immune system); BRCA1 and BRCA2 (DNA repair genes); ATM (DNA repair); TET2 (telomeres); SH2B3 (inflammation); CHRNA3, CHRA5, CELSR2, and PSRC1 (intercellular communication); NF–kB (inflammation); and USP42, TMTC2, and CLU (protein housekeeping). Those are all nuclear DNA variants. There are also many in mitochondrial DNA that are associated with human lifespan, such as the mechanistic target of rapamycin (*mTOR)*, *CPS1*, *MFN2*, and *LRPPRC* that are involved with mitochondrial function and metabolism.

All that's interesting, but what's actionable? An important exome sequencing study in nearly fifty-eight thousand Icelanders brought these investigations to a practical realm. This unique study tied the genomic data—from just fifty-three actionable genes—to the country's death registry. Four percent carried a disease-causing mutation that changed their lifespan, such as the LDL receptor (6.5 years reduced lifespan), and various cancer mutations (BRCA1, BRCA2, MSH2) were associated with three years of reduced lifespan. The main point is that genomic data at the individual level for one in twenty-five people provided actionable information affecting their lifespan. That 4 percent level of finding important disease-causing mutations has been replicated in several other studies. As pointed out in chapter 6:

> By the age of 65 years, 10 percent of the participants with pathogenic or likely pathogenic variants in cancer genes had died. In contrast, in the group of participants without such a variant, 10 percent had died by 73 years of age.

You'll note the lack of overlap for most of the actionable genes here, which are disease causing, compared with the genomic underpinnings of the aging process, not actionable now, but some of which may be addressed with drugs or ultimately genome editing in the future.

A polygenic risk score for common variants linked to lifespan was established in nearly seven hundred thousand multiancestry participants and in the UK Biobank separately. Common variants in lipid metabolism genes like LPA, for which medications are emerging; TP53, a DNA repair and when mutated a major cancer predisposition gene; and many others that are linked to risk of heart disease or cancer have been uncovered. Having a polygenic risk score is helpful to dissect the relative contribution of genetics and lifestyle to longevity.

Everything so far mentioned on genomics refers to germline DNA, what we're conceived with. But there's another group of genomic mutations that occur during life known as somatic mutations, in our body, not our germline. We've learned a lot about these variants from single-cell sequencing: we're all mosaics! When we age, we accumulate DNA mutations throughout our tissues and cells; therefore, the sets of genes in the cells of our body are far from uniform. We are mosaics of slightly varying genetic codes. And it's not just aging that does this.

Our exposures to environmental factors such as UV light or exogenous chemical agents can cause genomic instability, and it's not confined to single letter changes or any particular cell type. We've seen the importance of jumping genes (transposons) unleashed, moving from one place to another in the three-dimensional genome, and mutations in stem cells that can alter the aging process.

A checkpoint for genes that are involved in the aging process is to see if evolution conserved them—that they are identical or similar across species. Indeed, many of the gene variants and pathways associated with human lifespan, or their ortholog counterparts, are seen across multiple species. That's notable given the marked variability in maximum lifespan as seen in figure 12.2.

That differs for somatic mutations and is known as Peto's paradox. Mammals that live longer accumulate mutations at a slower rate. Those mammals also tend to be larger, with considerably higher cell mass, such that this slower mutation rate may have evolved to protect against cancer. Within many species that have been assessed, smaller size has an associated advantage for longer lifespans.

Especially intriguing is the profound slowing of aging in turtles, twentyfold slower than mammals and twice as slow as humans and birds. There is intense interest for learning more on the aging process through dogs, with a large open science Dog Aging project and UK Dogs Trust initiative. But all these vertebrates can't compete with the immortality of jellyfish! Whole genome sequencing of the immortal jellyfish *Turritopsis dohrnii*, the only species to rejuvenate repeatedly after sexual reproduction, has demonstrated gene variants that are connected with most of the hallmarks of aging, including genomic instability, telomeres, mitochondrial dysfunction, stem cell exhaustion, intercellular communication, and cellular senescence.

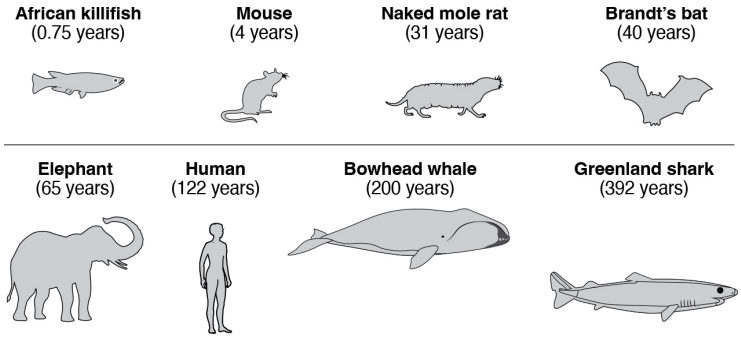



***Figure 12.2.*** Maximum lifespan of multiple species. Adapted from Param Priya Singh et al., "The genetics of aging: A vertebrate perspective," *Cell* 177, no. 1 (March 2019): 200–220, https://doi.org/10.1016/j.cell.2019.02.038. Description 57

## Telomeres

Throughout life there is a steady erosion to the tips of chromosomes, an attrition that is part of normal aging and consistent across all chromosomes of an individual. Once a cell's telomeres get too short, it can no longer divide. But it's a Goldilocks story since long telomeres (adjusted for age) predispose to cancer and short ones are linked to age-related diseases. The long telomere story is much akin to longer lifespan across species, promoting vulnerability, but here with a faster rate of accumulation of mutations. Many types of common cancers, and especially malignant melanoma, are seen at increased rates with long telomeres. There are adult-onset inherited short telomere syndromes that include premature hair graying, idiopathic (cause unknown) pulmonary fibrosis, and T cell immunodeficiency. The latter increases the risk of hematologic cancers such as myelodysplastic syndrome and acute myelogenous leukemia. In the mouse model, activation of telomeres by telomerase gene therapy inhibited pulmonary fibrosis. The recent discovery of a telomerase verse transcriptase activator compound is encouraging because it promoted telomere synthesis, blunted tissue aging hallmarks, and reduced brain inflammation, all without risk of cancer, in the mouse model.

Short telomeres also predispose to the accumulation of mutations in blood stem cells. This condition, known as clonal hematopoiesis of indeterminate potential (CHIP), is increasingly common with age and is associated with a higher rate of cardiovascular disease, blood clotting events, and chronic liver

disease. The most common driver mutation in the blood cell clones is in DNMT3A, a gene that controls telomere length, which tells us how tightly CHIP and telomeres are intertwined. These heightened risks are believed to be due to promoting inflammation or the process of "inflammaging," as documented in experimental models. Over 10 percent of people aged seventy and older have CHIP, and I have argued that the *I* for "indeterminate" now be dropped because these blood stem cell mutations carry a clear risk; it's no longer fuzzy. I believe this condition should be routinely assessed in older adults as part of a comprehensive risk assessment to be integrated with all the multilayered data we've been reviewing throughout the book.

## Epigenetics

Epigenetics is all about our DNA 3D packaging, which can be broken down to three principal components: methylation, histones, and chromatin, collectively giving this packaging immense power to turn our genes on or off. As we age, our DNA gets methylated. In other words, a methyl group is added to specific cytosine nucleotides of DNA, known as CpG methylation, which can change gene expression. Our DNA also experiences other changes: our histones get acetylated, and our chromatin undergoes unfavorable remodeling. Typically, DNA methylation leads to a gene's silencing, while histone acetylation leads to its activation. The epigenome's relationship with aging seems bidirectional: it plays a major role in both regulating it and being modulated by it. With all our twenty thousand genes potentially impacted by DNA methylation and changes of histone or chromatin, along with noncoding RNAs, one would expect the epigenome to have some interaction with most, if not all, of the twelve hallmarks of aging—and it does.

Of the epigenetic components, DNA methylation is the easiest to measure. Researchers have developed tools, known as epigenetic clocks, to predict biological age. These clocks measure the accumulation and pattern of DNA methylation to determine the biological age of a cell or tissue, such as the blood, which provides a comparison of biologic versus chronologic age. If the blood cells are biologically older than chronologic age, that is an indicator of accelerated

aging. The functionality of these clocks was validated in 2011 and has continued to be more precisely defined so we can more accurately estimate biological age. We can say more about these clocks and biomarkers of aging, but for now, the fact that methylation has been relied upon as a key way to track the aging process reinforces the pivotal role of epigenetics. The maximal lifespan across 348 mammalian species (partly represented in fig. 12.2), including humans, is predicted by epigenetic clocks. A clever analysis using the technique of Mendelian randomization of DNA methylation data was used to support its cause-and-effect relationship with health span and longevity.

That was brought home by my friend Juan Carlos Izpisua Belmonte, previously at the Salk Institute and more recently moved to Altos Labs, who made a chain of remarkable discoveries about epigenetic alteration, how it rejuvenated cells and whole animals, culminating in a seminal paper published in 2016. We gave him our Scripps Research Award in 2018 for the Future of Genomic Medicine, but it took years before the impact of this extraordinary work of rewinding time at the whole organism level was fully appreciated. Belmonte and his team used the four Yamanaka gene transcription factors (Oct3/4, Klf4, Sox2, c-Myc, abbreviated OKSM) named after Shinya Yamanaka, the stem cell scientist who won the Nobel Prize in 2012 for discovery of reprogramming an adult cell back to a pluripotent stem cell (known as induced pluripotent stem cells, iPSCs). When reprogramming is achieved, all the epigenetic marks are erased.

But with *partial reprogramming*, as pioneered by Belmonte's team at the Salk Institute, instead of forming pluripotent stem cells, just the aging epigenetic signatures are removed and the cell's identity is preserved. In this way, for example, old heart muscle cells would become young ones, but still were heart muscle cells. The partial reprogramming was then used to extend healthy aging and lifespan of prematurely aging mice. But achieving partial rather than complete reprogramming is the major challenge. One strategy is to use a brief OKSM exposure time. Fine-tuning this transient perturbation of the transcription factors can be thought of as adjusting a "volume control knob" to get the optimal setting. Otherwise, loss of identity of cells would ensue with potential transformation to cancer.

Further spotlighting the prominence of epigenetics with aging, Belmonte used CRISPR genome editing that targeted histone marks, instead of standard genome editing directed to DNA, achieving improved outcomes in mouse models of type 1 diabetes, muscular dystrophy, and acute kidney injury. Many subsequent studies from Belmonte and independent labs have replicated the potential for rejuvenation via epigenetic reprogramming, including animal organ studies of the heart, liver, eye, nerve fibers, pancreas, skin, and skeletal muscle. There is also a way to sidestep the Yamanaka factors with chemical reprogramming. Back in 2013, Hongkui Deng and his group in Beijing, China, discovered an alternative way of reprogramming to achieve pluripotent stem cells using a cocktail of small molecules. More recently, like Belmonte's team, they have adopted this small molecule epigenetic reprogramming to rejuvenate cells. Although there has been less work (in vivo) in experimental models, this is a distinctly different and efficient way of reprogramming and may prove to be a useful strategy. It avoids the problems of viruses and gene therapy and is potentially considerably cheaper.

## Protein Housekeeping (Proteostasis) and Cellular Garbage Removal

The loss of proteostasis with aging can lead to misfolded or aggregated proteins, as commonly seen with cataract formation, and as an underpinning of neurodegenerative diseases, including Parkinson's and Alzheimer's disease. The cell's ubiquitin pathway helps package the proteins that need to be discarded through the proteasome waste disposal system. Garbage removal is one of the cells' most vital and elaborate functions, not just for getting rid of misfolded proteins or ones that were improperly made. Inside the cell, the waste disposal process depends substantially on an organelle called the lysosome, which collects dud proteins and mitochondria into a kind of garbage bin. In the cell cytoplasm, there are autophagosomes, which transport these garbage bins, functioning as garbage trucks ridding the cell of waste products. This cleaning process, called autophagy, gets less efficient with age. Eventually the accumulation of waste and the bursting of lysosomes and mitochondria induce significant inflammation and

oxidative damage to cells and tissue. Many compounds that activate autophagy have been linked to longer lifespan in animal models, such as metformin or rapamycin. Caloric restriction and physical activity also help promote cellular house cleaning.

## Nutrient Sensing and Mitochondrial Dysfunction

Here's where growth hormone, glucose, insulin, IGF1, IGF-1R (insulin growth factor-1 receptor), amino acids, and the entire nutrient network that regulates so many cellular functions come into play. It feeds the powerhouses of the cell, the mitochondria. As I reviewed in chapter 3, there's been intensive study of caloric restriction in animal models, which in rodents, yeast, and fruit flies support lifespan extension with marked reduction of about 40 percent of daily caloric intake. However, the evidence in nonhuman primates is mixed, with lack of replication of lifespan extension. In humans, function of the thymus, a key regulator of our immune system, was improved with a 14 percent caloric restriction diet (considerably easier than the restriction levels in the animal model studies) for two years, along with various salutary metrics of reduced inflammation and improved mitochondrial function. Likewise, two years of mild (12%) caloric restriction significantly reduced biological age in more than seventeen hundred participants. In experimental models, various manipulations of the cell's ability to respond to fuel substrates, such as modulated by growth hormone or IGF-1 inhibition, a ketogenic diet, sirtuin activators, and mechanistic targets of rapamycin (mTOR), have been shown to have functional consequences on mitochondrial function, inflammation, autophagy, and cellular senescence. Mitochondrial-derived vesicles, which transport cargo inside the cells to lysosomes and other organelles, are increasingly recognized for their role in the aging process. It makes sense; if you can't process nutrients, you aren't going to be as healthy.

## Senescent Cells

In contrast with young, healthy cells, senescent cells have multiple distinguishing features, including larger size, increased size and number of mitochondria and lysosomes, inability to divide that leads to growth arrest, shortened telomeres, and richness in the enzyme beta-galactosidase (and change to a secretory phenotype). Old cells are not necessarily categorized as senescent—there is a biological shift that occurs in some of them that leads to their properties. And senescent cell features vary considerably, so they shouldn't be thought of as a simple categorization. Their accumulation promotes chronic inflammation, which in turn, as a stressor, induces more cells to become senescent. These cells function as bad actors beyond the aging process, linked to cardiovascular, brain, kidney, liver, musculoskeletal, and endocrine diseases. Senescence of immune cells leads to decreased function, less antibody production, and increased susceptibility to infections. A strong case for the senescence of neurons as a driver for brain aging has been made. But all senescent cells aren't bad. There is considerable tissue and cell specificity, whereby some types of senescent cells promote healthy functions such as wound healing, tumor suppression, pancreatic beta cell insulin secretion, and tissue repair. Nonetheless, nonselective elimination of senescent cells promotes lifespan in mice, and in the antiaging clinical trials section to follow I'll address the many drug "senolytic" efforts that target old cells.

## Exhausted Stem Cells

Adult stem cells vary by tissue, such as blood (hematopoietic, HSCs) or muscle satellite cells (MuSCs). Wherever they are, they are pivotal to health, owing to their self-renewal capability. Stem cells are endowed with long life, making them particularly vulnerable to cell damage that occurs with aging. One of the ways this is manifest in older adults is sarcopenia—loss of muscle mass and strength—since MuSC function is impaired, with less ability to repair or regenerate skeletal muscle cells, and chronic activation of pro-inflammatory pathways leading to exhaustion. MuSCs can, in part, be rejuvenated with exercise. Similarly, for blood

stem cells, aging results in reduced fitness and function, and an antibody that depletes exhausted HSCs in mice rejuvenates the immune system. These examples provide strong support that stem cells are not only a part or bystander of the aging process but also a driver.

## Intercellular and Interorgan Communication

How cells talk to one another within tissues and long range is altered by aging, with the exemplar being a phenotype of senescent cells termed SASP, the senescent cells capable of secreting various soluble factors, growth factors, and extracellular matrix–remodeling enzymes. Aged immune cells are not only in close communication with one another but also signaling throughout the body. Inflammation of our gums has been recognized as a risk factor for heart arrhythmias in the form of atrial fibrillation. In an experimental model, stressed mitochondria from microglia cells in the brain were shown to communicate with cells throughout the body to promote repair. Notably, in developing the muscle cell atlas of aging, researchers created "CellChat" to wiretap cell-to-cell interactions that occur through elaborate circuits. They observed that certain interactions doubled with aging, particularly ones that promote the muscle-wasting process through inflammation, dysregulation of growth factors, and excessive deposition of extracellular matrix. This just scratches the surface for the intricate crosstalk occurring between cells that is strongly influenced by the aging process.

A series of recent studies indicates that chatter between organs promotes health and in experimental models extends lifespan. Muscle tissue sends out six hundred molecular signals, fat more than one hundred, which are widely interacting with other organs, not just via circulating blood. For example, activating the communication between the hypothalamus and fat tissue enhanced physical activity and improved lifespan in the mouse model. This occurred via the sympathetic nervous system, mediated by fat cell secretion of the signal extracellular nicotinamide phosphoribosyl-transferase. When these extracellular vesicles from young mice are infused into old ones, lifespan is extended. Bone is another important source of chatter, and its osteocalcin signal promotes liver

release of insulin and muscle absorption of glucose, and improves cognition. Another example of organ-organ crosstalk comes from the heart, which has a stress memory, leading to epigenetic marks on blood stem cells, setting up recurrence of heart failure and damage to kidney and skeletal muscle in the mouse model. So many of our body-wide interorgan circuits are getting redrawn, providing further clues on how to modulate the aging process in experimental models.

## Chronic Inflammation

Inflammaging is the process of systemic inflammation with aging, but it is highly variable from one individual to another as measured by C-reactive protein, interleukin-6, tumor necrosis factor-α (TNF), and latent virus infections. More extensive and durable chronic inflammation has been linked to promoting cancer as well as neurodegenerative, cardiovascular, and metabolic diseases. In the aged mouse model, interleukin-11 (IL-11) inhibition, which works in the interleukin-6 pathway, had a broad effect of improving metabolism and muscle function, blocking tumors, changing white fat to beige fat, and significantly expanding lifespan, adding more support for inflammation as a driver of aging, and as a possible intervention since this drug is already in clinical trials for interstitial lung disease. The potential to combine this with an interleukin-6 blocker, which has suppressed heart disease and cancer in a large randomized trial, is alluring. There is a way to do just that. Much of the inflammation unleashed by the activated NLRP3 inflammasome,[I] a large protein complex of our innate immune system, can be blocked by small molecules (pills) that inhibit multiple key pro-inflammatory proteins including interleukin-1β and interleukin-6. There are many other potential drivers of untoward inflammation that are not yet unraveled, but the cGAS-STING pathway (comprised of cyclic GMP-AMP synthase [cGAS]), stimulator of interferon genes (STING), in the cell cytoplasm, has been implicated as a major mediator. It is activated by cell damage, such as sensing DNA leak from mitochondria. When this pathway is blocked, age-related inflammation of multiple organs and the brain is reduced.

### Gut Microbiome

The critical role of the gut microbiome in modulating our immune response has been emphasized throughout the book, but it extends from that into the whole aging process. Its composition differs in centenarians with youth-associated profiles of bacteria and viruses. In a study of more than nine thousand individuals, a microbiome pattern, such as depletion of *Bacteroides*, was associated with healthy aging. A gut microbial age metric was derived from more than ten thousand people aged forty to ninety-three years and correlated with the risk of cardiovascular disease over extended follow-up. The gut microbiome was profiled for correlation with frailty in more than eighteen hundred older adults, with specific bacterial species linked to high frailty scores and to clinical outcomes over the next two years. Fecal transplant of the gut microbiome from young mice to old mice improved cognitive behavior. In the opposite direction, transplants from old to young mice markedly promoted inflammation and inflammaging. These and many other experiments anchor the gut microbiome as a key player in the aging process.

Now that we've briefly reviewed the twelve biological hallmarks of aging, we're ready to zoom in on the biomarkers that can be used to quantify our age.

## BODY-WIDE BIOMARKERS OF AGING

The first epigenetic predictor of age, published back in 2011, could predict a person's chronological age to within five years. It used saliva samples in thirty-four twin pairs assaying 88 CpG sites for DNA methylation. Recall that CpG sites are methyl groups attached to cytosine nucleotides. Two epigenetic clocks were published a couple of years later, one by Gregory Hannum and colleagues using blood in nineteen people with 71 CpG sites, and Steve Horvath (a coauthor of the two other preceding papers) using 353 CpG sites in multiple tissues for a clock, coined DNAmAge, that has "a high tick rate until adulthood, after which it slows to a constant tick rate." These epigenetic clocks have been further refined over the years, with three major ones now being used extensively in research (DNAm PhenoAge, DNAm GrimAge, and DunedinPACE). The DNAm

PhenoAge clock predicts biological age from DNA methylation, chronological age, and clinical parameters (including lab tests of albumin, creatinine, glucose, C-reactive protein, lymphocyte percent, and white blood cell count) associated with mortality risk. Among almost 150,000 people from the UK Biobank, there was a higher PhenoAge in people born after 1965, such that their biologic age was outpacing their chronologic age.

That is an alarming finding. This observation of cells aging faster across the population has been offered as a potential explanation for the increased incidence of cancer in young people. PhenoAge has also been shown to predict cardiometabolic diseases and mortality. The DNAm GrimAge publication entitled "GrimAge strongly predicts lifespan and healthspan" uses a two-stage approach, multiple blood biomarkers, and added data inputs such as smoking pack-years to differentiate slow versus fast aging. Subsequently, the DunedinPACE clock was derived from DNA methylation and nineteen biomarkers in blood for determining pace of aging. It uniquely uses longitudinal data from a large birth cohort unlike the others that rely on cross-sectional data from different cohorts. The methylation markers in epigenetic clocks can be used to assess population-wide impact such as the COVID pandemic and smoking, with patterns indicative of accelerated aging. The loss of a family member's life has been associated with acceleration of epigenetic clocks; exercise and a plant-based diet have been associated with slowing the pace. But the different epigenetic clocks differ in their predictive accuracy and response to various interventions, which has resulted in the need for benchmarking studies, currently a work in progress.

Many other clocks have been built from other omic layers, including metabolomics, transcriptomics, proteomics, lipidomics, and glycomics (measuring the sugar attachments to proteins). Of these, a proteomic aging clock has had extensive validation across three country biobanks (UK, Finland, and China) for its association with the risk of eighteen chronic diseases, mortality, and multiple age-related traits such as cognitive decline. This landmark study emphasized how important blood proteins will be in the future for predicting age-related functional status.

An inflammation marker clock (iAge) has been shown to reflect biologic age, as has the immune system response (IMM-AGE). There are also "mitotic cell" division clocks, which include both global DNA hypomethylation and focal hypermethylation specifically related to cancer risk. A multimodal AI model that integrated images of face, tongue, and retina from more than eleven thousand healthy participants, without any blood tests, also purported to provide an accurate estimation of biological age. ExplaiNAble BioLogical (ENABL) Age is another AI multimodal model that used genomics, protein markers, and clinical history in three large and diverse populations, with improvement for interpretation of biological age and accuracy of mortality prediction compared with PhenoAge and GrimAge. EyeAge uses deep-learning AI of the retinal image to determine chronological age and was more accurate than epigenetic clocks in three different population cohorts. A problem with most of these models is that chronic diseases are in the mix, so we don't see age-related illness versus the healthy aging process. One model with the specific objective to disentangle these two strands found that a low neutrophil count and low alkaline phosphatase on lab tests at ages fifty to sixty years were predictive of healthy aging, well beyond seventy years.

We're at a time when thousands of plasma proteins can be assayed in thousands of people at different ages, enabling proteomic clocks and, as we'll get into momentarily, organ clocks. When this was done to elucidate the aging proteome, three distinct peaks were found (fig. 12.3) across the human lifespan, each with different sets of proteins. The second peak at middle age was predictive of cardiovascular disease and Alzheimer's. I think of Ernest Hemingway's character Mike's line in response to how he went bankrupt in *The Sun Also Rises*: "Gradually, then suddenly." Aging proteins in contrast speed up and slow down at certain points. These clocks may be providing the vital warning signs to head off the later, sudden demise.

But do these biologic omic clocks, beyond the second-generation epigenetic clocks, provide unique or complementary information about the aging process? That's uncertain. A study that assessed genomics, proteomics, and metabolomics, but not epigenetics, in more than thirty-five hundred individuals missed the opportunity to shed light on this uncertainty. The various clocks have been

developed from populations and have questionable relevance and specificity at the individual level. Beyond these unknowns, we really don't know what "biological age" means, especially for a given individual. While it's gratifying to get a report that your biological age is ten years younger than your chronological age, it doesn't have adequate anchoring from prospective clinical trials. For example, *if* the biologic age was accurately determined for a person to be several years greater than the chronological age, would knowledge of the clock result change lifestyle factors that have been associated with improvement? Or shouldn't that person be adapting the healthiest known lifestyle factors anyway? Curiosity is one thing, but right now all we have to offer are the lifestyle+ factors reviewed in chapter 3.

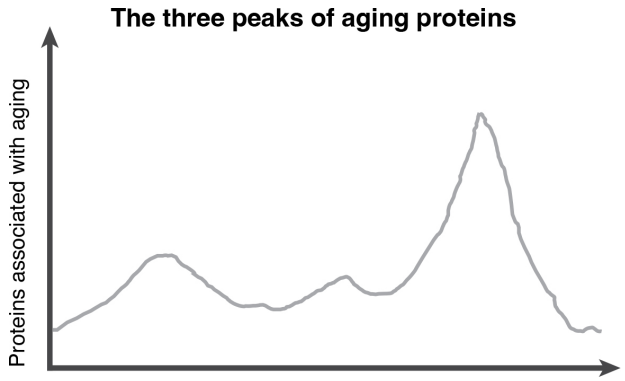



***Figure 12.3.*** The three distinct plasma protein peaks across the human lifespan. Adapted from Valur Emilsson et al., "Predicting health and life span with the deep plasma proteome," *Nature Medicine* 25, no. 12 (December 2019): 1815–16, https://doi.org/10.1038/s41591-019-0677-y. Description 58

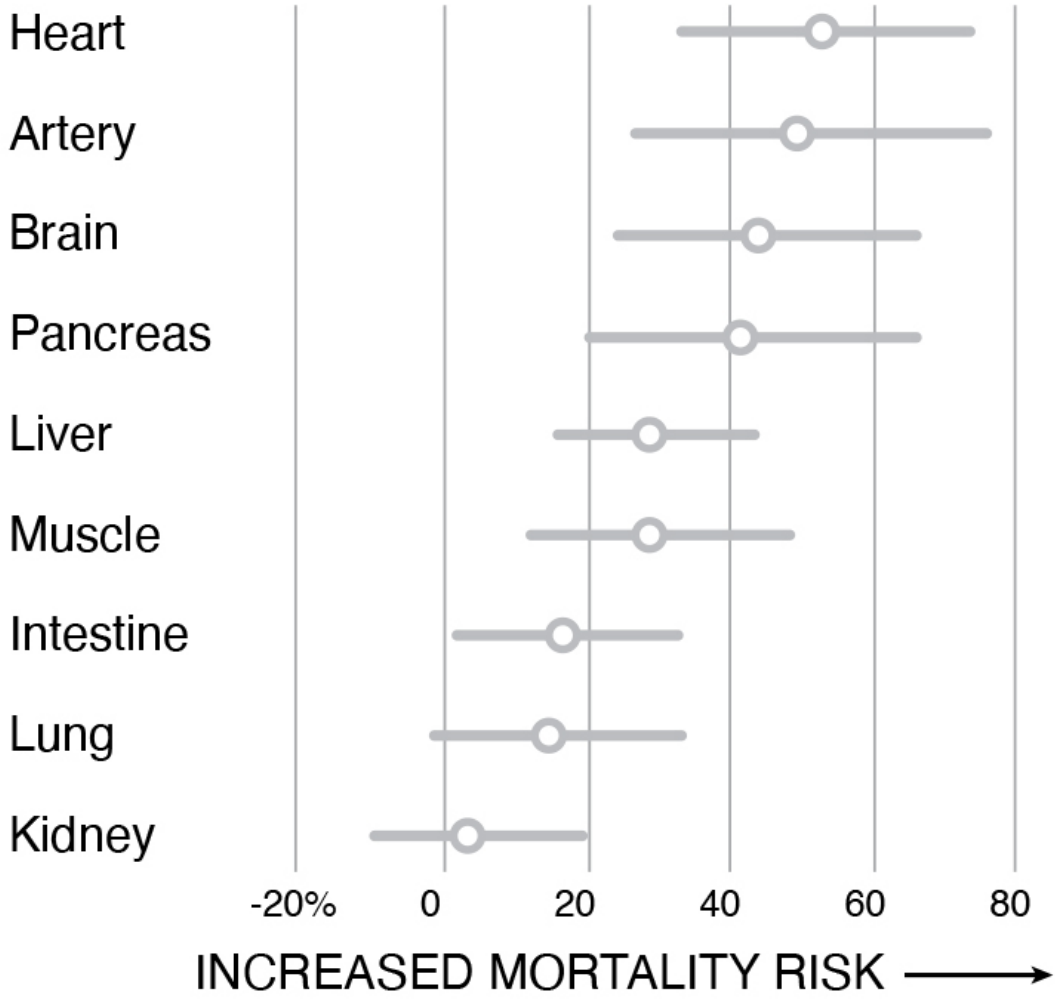
That hasn't held back commercial offering for aging clocks, such as the TruDiagnostic that provides the DunedinPACE result and metrics for immune cell function, "fitness," telomere length, and other reports; GlycanAge ($348 for one test, $599 for two); Generation Lab (four biological age tests for $149 per month and a VIP program for $3,499 per year). Companies including Tally Health, Elysium Health, and Novos Labs offer aging clocks and supplements to pair with them. I don't recommend any of these tests (or supplements), including telomere length and epigenetic clocks. They are useful in research studies, but there is lack of clinical validation from rigorous randomized trials that having some body clock information leads to improvement in metrics or health outcomes.

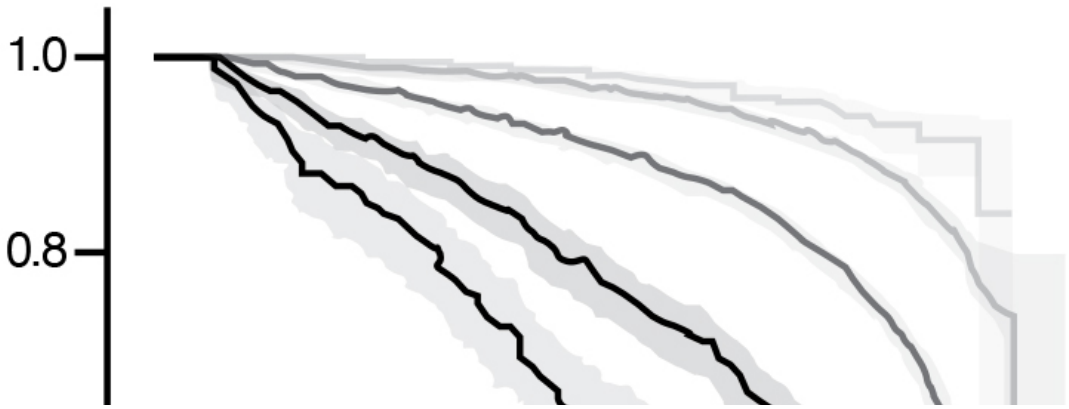
## ORGAN CLOCKS

The problem with the body-wide clocks is that some people are heart agers, while others are brain or kidney agers. Tony Wyss-Coray of Stanford University investigated this, assessing nearly five thousand plasma proteins for eleven organs in 5,676 people (from five independent cohorts) across the human lifespan. The study confirmed that our intrabody aging by organ and system is quite heterogeneous. Unique protein clusters tracked with each organ. One in five individuals had at least one organ in age acceleration, but only about 2 percent of people were multiorgan fast agers. Each organ's markers were predictive of adverse health outcomes over fifteen years of follow-up in the same domain, such as heart aging and a fivefold rate of heart failure or brain aging markers predicting a higher risk of Alzheimer's disease. Muscle aging predicted impairment of gait, while kidney aging predicted hypertension and the metabolic syndrome. The gap from organ age to chronological age of the person was associated with increased mortality during the extended follow-up period (fig. 12.4 top panel).

These findings were subsequently replicated and extended in two studies using the UK Biobank resource. In nearly fifty-three thousand UK Biobank participants, with measurement of three thousand plasma proteins, organ-specific aging clocks and impact of interventions were identified. For example, alcohol accelerated aging of the brain, kidney, and intestine. The effects of smoking were across the board—not just lung but also brain, immune system, liver, kidney, and intestine. Men were biologically older and tended to age faster than women across the various organs. Foods like muesli, oily fish, and raw salad were associated with slower multiorgan aging, whereas it was the opposite for sugar-sweetened beverages, white bread, and savory snacks. Ludger Geominne of Harvard Medical School and colleagues concluded the following: "These findings suggest that future antiaging interventions should not only target organismal aging, but could also be tailored towards the oldest organs, calling for a more personalized approach towards anti-aging interventions."

**Relationship between organ age gap and future mortality risk over 15 years**



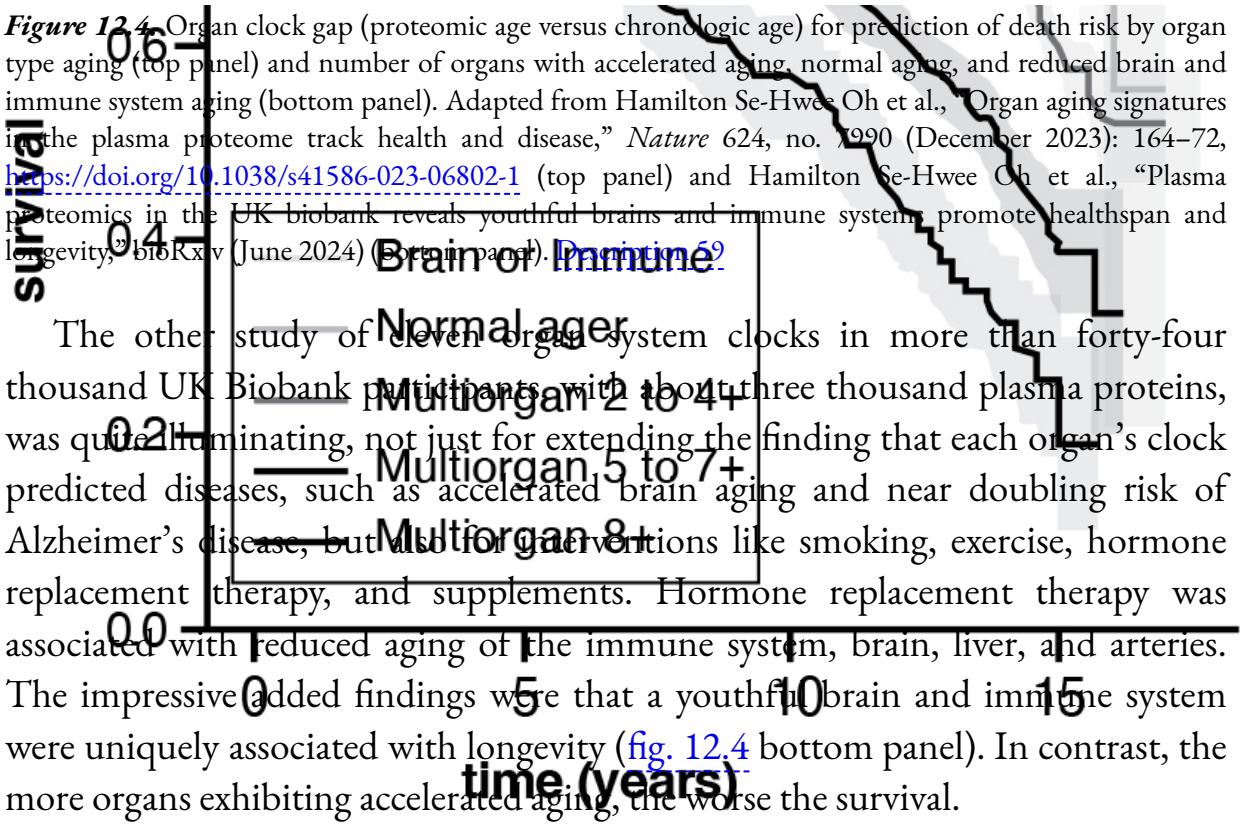



***Figure 12.4.*** Organ clock gap (proteomic age versus chronologic age) for prediction of death risk by organ type aging (top panel) and number of organs with accelerated aging, normal aging, and reduced brain and immune system aging (bottom panel). Adapted from Hamilton Se-Hwee Oh et al., "Organ aging signatures in the plasma proteome track health and disease," *Nature* 624, no. 7990 (December 2023): 164–72, https://doi.org/10.1038/s41586-023-06802-1 (top panel) and Hamilton Se-Hwee Oh et al., "Plasma proteomics in the UK biobank reveals youthful brains and immune systems promote healthspan and longevity," bioRxiv (June 2024) (bottom panel). Description 59

The other study of eleven organ system clocks in more than forty-four thousand UK Biobank participants, with about three thousand plasma proteins, was quite illuminating, not just for extending the finding that each organ's clock predicted diseases, such as accelerated brain aging and near doubling risk of Alzheimer's disease, but also for interventions like smoking, exercise, hormone replacement therapy, and supplements. Hormone replacement therapy was associated with reduced aging of the immune system, brain, liver, and arteries. The impressive added findings were that a youthful brain and immune system were uniquely associated with longevity (fig. 12.4 bottom panel). In contrast, the more organs exhibiting accelerated aging, the worse the survival.

In a UK Biobank cohort of nearly forty-two thousand participants, the assay results for three thousand plasma proteins were used to predict the subsequent occurrence of over two hundred common and rare diseases during a ten-year follow-up. Similarly, this high-throughput proteomic approach, with over twenty-nine hundred blood proteins, integrated with genomic and electronic health record data and analyzed with machine learning, was applied to a prospective cohort of more than twenty-five thousand participants showing predictive power across most diseases. Other plasma protein profiles have been used to predict dementia more than a decade before diagnosis. A study of more than fifty-two thousand healthy adults with over fourteen hundred plasma proteins found four (GFAP, NEFL, GD15, and LTBP2) that were highly specific for dementia prediction. A plasma protein risk score may help to predict hip fracture, a major age-related condition with a high fatality rate.

If validated for actionability at the individual level for improving health outcomes, I would consider the plasma protein organ clocks a major advance for tracking the aging process. By providing specificity of risk at the organ level for an individual, this would be paired with actionable steps known to decelerate aging

—the right information about the right organ in the right person—exemplifying preventive individualized medicine. Further, organ clocks may be especially useful for regulatory authorities as a surrogate metric for a drug's specific therapeutic antiaging effect. Overall, resetting an organ clock in a person is a less ambitious, more attainable goal than trying to do so at the whole-body level.

## TISSUE-SPECIFIC AGING

Much of the aging process is tissue specific. To understand why some people aged eighty and older are super brain-agers, compared with age-matched controls, comprehensive assessment revealed more brain gray matter volume, slower gray matter atrophy, faster movement speed, more preserved white matter microstructure, and better mental health. Notably, there was no difference in APOE4 carriers, amyloid accumulation in the brain, or lifestyle factors between groups. In a similar study, the super agers' brains had the look of fifty- to sixty-year-old brains instead of their eighty-year-old chronological age, as did their memory function. These reports provided structural brain differences and correlation with cognitive function, but they didn't help explain why some people have such a slow brain-aging process. Of interest is the finding of a markedly different trajectory of brain aging from tissue methylation (known as HistoAge clock) compared with the Horvath and related whole-body epigenetic clocks, which aligns well with the concept of organ- (and tissue-) specific aging patterns. So does a study of over four hundred omic features in more than four thousand people that showed again that organs are aging at very different rates within individuals.

The mounting evidence supports a "middle-aging" brain, like the second wave of plasma proteins of aging, when there is a turning point for multiple processes (fig. 12.5) that can be tracked, and for which there are some modifiable risk factors. This long period, ten to twenty years before neurodegenerative disease takes hold, provides a big opportunity to change the course of a person's natural history of cognitive function decline.

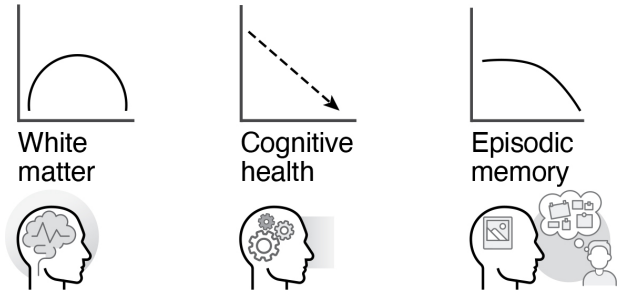



***Figure 12.5.*** "Middle-aging" brain changes. Adapted from Sebastian Dohm-Hansen et al., "The 'middle-aging' brain," *Trends in Neurosciences* 47, no. 4 (April 2024): 259–72, https://doi.org/10.1016/j.tins.2024.02.001. Description 60

Other health factors that can accelerate brain aging were shown in a recent report that chronic knee pain from osteoarthritis, assessed by MRI and memory function, affected the hippocampus, thalamus, and several regions of the brain and manifested increased risk of dementia during follow-up.

After the brain, muscle has perhaps been the most extensively studied organ for impact of aging, with the advantage of being able to acquire healthy tissue across the lifespan. Muscle biopsies from seventeen participants aged twenty to seventy-five years enabled sequencing of more than ninety thousand single cells and single nuclei. Insights from this work told us about stem cell dysfunction, increased pro-inflammatory protein production, activation of the immune system, and loss of nerve supply.

For heart aging, studying macaques indicates that heart muscle cells get much bigger with age, twice the size as young monkeys', along with increased production of pro-inflammatory proteins, fibrosis (scarring), and senescent cells. Somatic mutations of heart cells accumulate, exhibiting deficiencies in DNA repair, more than age-related changes in brain cells or lymphocytes. The biologic aging process of each organ, such as the brain, muscle, or heart, representative of other organs, can march to its own pace within a given individual. We also know, from compelling genetic evidence of interconnectedness, that "no organ system is an island."

## MODULATING THE AGING PROCESS

How might the aging process be influenced? There are two basic categories: lifestyle+ and pharmacologic interventions. Let's start with lifestyle.

Some studies look at the overall impact of a healthy lifestyle that includes diet, physical activity, and sleep. In more than 350,000 UK Biobank participants followed for more than ten years, a polygenic risk score for lifespan was used to tease out the genetic component. For lifespan, genetics appeared to contribute about 20 percent, and a favorable lifestyle had an independent pronounced effect. Mitigating the genetic risk of a shorter lifespan, lifestyle added about five years of life. In a smaller study of more than eleven thousand individuals from four cohorts with a much longer follow-up of twenty-eight years, a healthy lifestyle metabolomic signature (predominantly lipid metabolism and strongly aligned with low BMI and healthy diet) was associated with a 25 percent increase in longevity (reaching age eighty-five and older) and a significant lower risk of all-cause mortality as well as cardiovascular and cancer mortality. The participants for both reports were predominately of white-European ancestry, limiting ability to extrapolate.

In a study of healthy lifestyle that followed twenty thousand participants in Japan over twenty-one years, there was considerable expansion of lifespan despite chronic conditions (diabetes, cardiovascular, kidney, cancer) even beyond those aged eighty years. A "dose-effect" was seen by the number of healthy lifestyles for diet, sleep, exercise, low alcohol intake, BMI, and no smoking (fig. 12.6). Similarly, in a study of nearly fifteen hundred Chinese centenarians, with matched controls of people who died before becoming centenarians, the highest healthy lifestyle score was linked to most likelihood of living to an average ninety-five years.

While caloric restriction has improved lifespan in rodents, the impact in nonhuman primates and people is less clear. Recall the contradictory evidence in the two large monkey studies with an over twenty-year follow-up, one by the University of Wisconsin and the other by the National Institute on Aging. The Wisconsin study was more restrictive of calories, with one meal a day and a sixteen-hour fasting period; the National Institute on Aging protocol left food

out overnight for ad lib intake. While lifespan was only extended in the Wisconsin study, both showed improvement in health span—age-related diseases of diabetes, cardiovascular disease, and osteoarthritis.

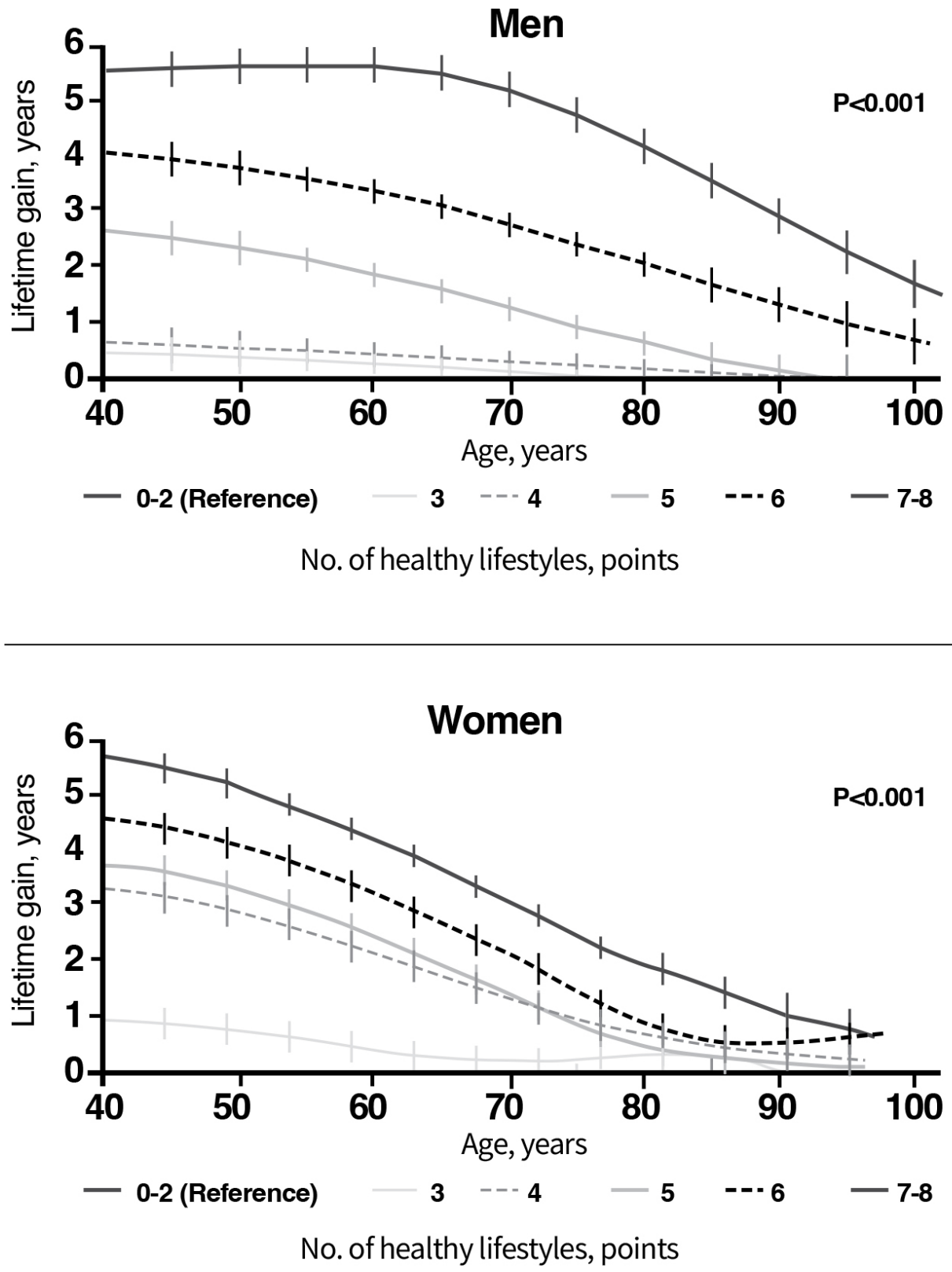



***Figure 12.6.*** Healthy lifestyle factors and gain in life years, by men and women. Adapted from Ryoto Sakaniwa et al., "Impact of modifiable healthy lifestyle adoption on lifetime gain from middle to older age,"

In the section on nutrient sensing, I mentioned the two studies that suggested mild caloric restriction (12–14%) was associated with reduced biological aging. A subsequent report from one of these studies provided a new dimension of caloric restriction's benefit—the immune system. Specifically, the thymus gland, which in adolescence diminishes in its ability to produce T cells and accumulates fat, was restored. Fat tissue was remodeled, and a reduction of a platelet-activating factor (PLA2G7) expression in that tissue was correlated with reduction of body-wide inflammation. The removal of that gene in mice provided evidence for blunting inflammation and preserving thymus function in old mice.

The various time-restricted eating and intermittent fasting practices discussed in chapter 3 have not provided support for antiaging impact but have shown improvement in the immune response. Along with caloric restriction, they are associated with favorable changes in the gut microbiome composition and better anticancer effects in experimental models. There has also been interest in a fasting-mimicking diet, a five-day intake of a plant-based, low-calorie, low-protein diet every four weeks, which showed beneficial effects in mice and was tested in a randomized trial of one hundred people (mean age forty-three years, BMI 27). Three five-day cycles of this diet led to better insulin sensitivity, less liver fat, an increase in lymphocytes to white blood cell ratio (a favorable immune system age metric), and a decrease of 2.5 years in biological age. The biological age metric was not based on DNA methylation but instead used a group of biomarkers. Valter Longo and Rozalyn Anderson, researchers based at the University of Southern California, have long been advocates of the fast-mimicking diet; in a review, they described the "longevity diet" for extended lifespan and health span as "characterized by a mid to high carbohydrate and low but sufficient protein intake that is mostly plant based but includes regular consumption of pesco-vegetarian-derived proteins."

On the other hand, a high-fat or ketogenic diet, particularly on a long-term basis, has been linked to accelerated aging in mice and humans, reflected by increased cellular senescence. Changes in the gut microbiome, and specifically an increase in the species of *Desulovibrio* bacteria, was found after a high-fat diet in mice with markedly increased leucine production. High blood leucine was also

seen in women with breast cancer with poor clinical outcomes. Experiments to untangle the high-fat diet and changes in gut microbiome revealed that leucine signaled the bone marrow to activate white cell suppressor cells, the mTOR pathway, and to promote tumor progression. Collectively, these findings and the adverse cardiovascular effects summarized in chapter 3 suggest that we should avoid long-term ketogenic diets.

While there are no dietary supplements that have been shown to promote health span, there is one good candidate—taurine. Taurine levels in people decline with age, and deficiency has been correlated with higher levels of inflammation and age-related diseases. Supplementation promoted health span in monkeys and reduced cellular senescence, inflammaging, and mitochondrial dysfunction. We await randomized trials to verify the effects in animal models.

Exercise has impressive effects for extending health span. Less inflammation, a fortified immune response, less atherosclerosis, better insulin sensitivity and less metabolic syndrome and type 2 diabetes, and a reduction in incidence of many cancers have consistently been seen in multiple reports. In a large study of more than 650,000 individuals aged twenty-one to ninety, with ten years of follow-up, a substantial gain in lifespan was seen with increasing metabolic equivalent hours per week (MET-hr/wk) of physical activity (fig. 12.7). Compared with no physical activity, this equated to about 4.5 years of life gained linked with 450 minutes of walking each week.

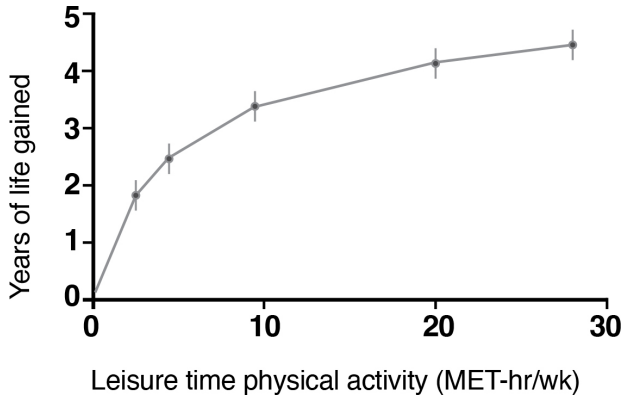



***Figure 12.7.*** Relationship between physical activity and years of life gained. Adapted from Steven Moore et al., "Leisure time physical activity of moderate to vigorous intensity and mortality: A large pooled cohort analysis," *PLOS Medicine* 9, no. 11 (2012): e1001335, https://doi.org/10.1371/journal.pmed.1001335. Description 62

As Steve Horvath and I reviewed, exercise is the only well-validated intervention to reset biological age in humans (fig. 12.8). Some of the salutary effects of exercise can be attributed to exerkines—the multiplicity of compounds that are released. While some of these tone down inflammation, like interleukin-10, others, like interleukin-6, can increase with high intensity and long duration, which simulates the stress response, an adaptive preconditioning for the real thing. An example is the immune response from one hour of aerobic and strength exercise in people with pancreatic cancer: not only a significant jump in CD8+ cytotoxic T cells but with a doubling of survival time for these cells to help fight the cancer. A big exercise NIH research Molecular Transducers of Physical Activity Consortium initiative began in 2016 in both animal models and more than twenty-five hundred people. Findings from the multiomic study of rats with treadmill training over eight weeks showed better mitochondrial function, less inflammation, likely reduction of autoimmune disease such as inflammatory bowel disease, and protection from fatty liver disease. In a separate experimental study, rejuvenation of skeletal muscle stem cells in old mice was achieved by exercise.

There are other lifestyle features that have been assessed for pro- or antiaging properties. Early exposure to tobacco, as a fetus or during childhood, has been linked to accelerated epigenetic aging. The stress from major surgery increases epigenetic aging, and social stress facilitates immune aging.

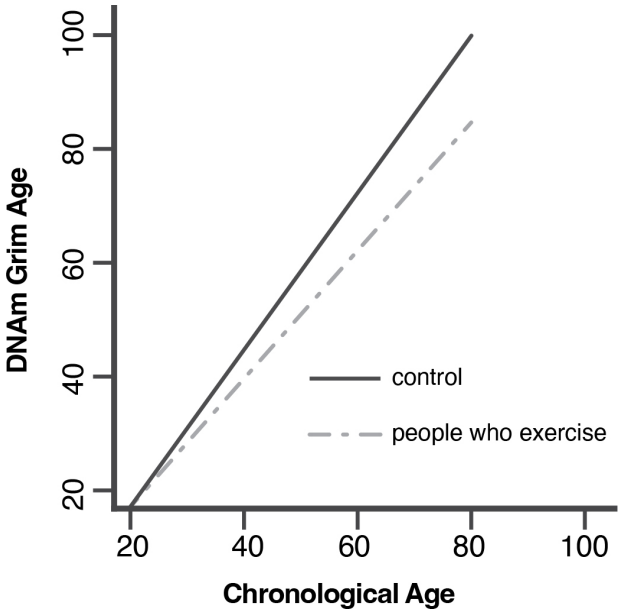



***Figure 12.8.*** Epigenetic aging clock favorably modified by exercise. Adapted from Steve Horvath et al., "Digitising the ageing process with epigenetic clocks," *Lancet* 404, no. 10451 (August 2024): 423, https://doi.org/10.1016/s0140-6736(24)01554-x. Description 63

The relationship of hobby engagement with healthy aging was studied in more than ninety-two thousand people aged sixty-five years and older in sixteen countries with a consistent link to improvement. That brings to mind Mrs. L. R. and her hobbies of painting and jigsaw puzzles at the healthy and robust age of ninety-eight years. As does her optimism, which has been associated with 11 to 15 percent longer lifespan. Add to that her lack of isolation and social connectedness. A recent study of more than 280,000 people using an AI-processed electrocardiogram biologic aging metric indicated that the least isolated people had the slowest biological aging.

As reviewed in chapter 3, with aging, our sleep quality and circadian rhythm are disrupted. Epigenetic aging can be slowed (using PhenoAge as the metric) with better sleep health. Perhaps more surprisingly, the built environment has been found to have connections with biological aging, such as air pollution from traffic or disadvantaged neighborhoods characterized by poorly maintained roads, abandoned cars, and nonart graffiti.

## INTERVENTIONS TO SLOW AGING

Modulating the aging process is all very well, but the prize many of us have in mind is actually reversing any functional declines. What can we say about that? Overall, the evidence is slight but intriguing, even exciting. Of course, I will start with rejuvenating the immune system.

We know by now how the age-induced changes to the immune system, or immunosenescence, are detrimental. In the animal model of defective DNA repair, immunosenescence can drive the aging of multiple organs, including the liver, kidneys, pancreas, and lungs. Through single-cell sequencing millions of immune cells of people at different ages, we're now learning precisely which cells are affected with the aging process.

Three very different approaches have been assessed to reverse aging of the immune system. A combination of recombinant human growth hormone, metformin, and dehydroepiandrosterone was given to a small group of ten male participants aged fifty to sixty-five years; only growth hormone has a known effect on the thymus gland and its vital importance to T cell production. MRI revealed evidence of thymus regeneration in the male participants. Compared with baseline, there was a decrease in multiple inflammatory markers and a heightened immune response. Epigenetic age (by Horvath and GrimAge clocks) dropped a mean of one and a half years, with further reduction at one year follow-up. A few words on growth hormone as a potential intervention: although levels decline with age and supplementation improves muscle mass and bone density in older adults, it promotes insulin resistance, and there are no data to support a favorable impact on lifespan.

In aged mice, use of a drug for four days that helps reorganize blood stem cells (countering the process known as polarization) led to reversal of the epigenetic clock and expanded lifespan. The same pronounced antiaging effects were seen with the drug given to immunocompromised aged mice that received transplanted rejuvenated blood stem cells. These blood stem cell rejuvenation experiments shifted the balance of the immune system toward lymphocyte rather than white cell production and helped set the foundation for the next report, which was published in *Nature*.

That approach involved antiaging antibodies in old mice. Antibodies directed to deplete aberrant, aged stem cells (known as myeloid-based hematopoietic stem cells), considered to be drivers of inflammation (inflammaging), shifted the immune cell population, from the white blood cells of the innate system to production of T and B cells, rejuvenating adaptive immunity for several months after a single injection. The enhanced immune system showed its advantage in response to viral infection of the old mice as compared with controls. The lingering concern, as with so many antiaging interventions, is that this rebalance of the immune system may present a liability for cancer, such as T and B lymphocyte cancers.

Let's return to my friend Juan Carlos Izpisua Belmonte and his seminal work in this area, which until now has been shown to rejuvenate mice genetically bred with the accelerated aging progeria gene mutation. When I visited with him recently, he told me about the dilemma for Altos Labs, which has attracted more than $3 billion of investments but has yet to initiate its first human clinical trial. Giving the Yamanaka factors systemically (throughout the body), even with a brief exposure, could promote cancer since two of the transcription factors can turn normal cells into cancer cells (c-Myc and OCT4). Teratoma tumors have formed in animals after exposure. Notably, Belmonte and his team have shown that short pulses of the four transcription factors, be they administered to the whole body or tissue targeted, avoided any cancer incidences in mouse models through twelve months. Perhaps instilling these factors into just one part of the body, such as a knee joint with severe degenerative osteoarthritis, might be a prudent initial step. In the eyes of old mice with glaucoma, giving three of the Yamanaka factors helped restore vision and reverse the epigenetic clock of retinal ganglion cells. Again, the concern is the trade-off between antiaging and the potential risk of cancer. By using short pulses of Yamanaka factors and a tissue-specific (and even damaged cell–specific rather than a whole-body) approach, the risk may be mitigated.

Another idea might be to attempt rejuvenation of the immune system in patients with terminal cancer. That would introduce the risk of cytokine release syndrome, which can be a fatal sequela for an unbridled immune response. Another possibility would be to specifically target thymus tissue, as was done

with growth hormone, but the method for such a precise delivery is not yet worked out.

For an alternative to growth hormone for antiaging immune cells, partial epigenetic reprogramming with the Yamanaka factors (OKSM) plus two accessory factors (LIN28 and NANOG) was undertaken in various types of cells in culture, including immune cells, to reverse epigenetic and inflammatory signatures. But this has not yet been tested in experimental models or humans. In the meantime, this may be used outside the body to rejuvenate engineered T cells used for cancer therapy.

There is an exceptionally large number of companies betting big on this strategy of partial epigenetic reprogramming, which includes not only Altos but also New Limit, Rejuvenate Bio, Life Biosciences, YouthBio Therapeutics, Shift Bioscience, Retro Biosciences, and several others.

Any search for a fountain of youth is bound to consider young blood. For more than two decades, we've known about its magic. Initial experiments involved the surgical union of two mice (parabiosis), one old and one young (heterochronic), with shared blood supply, providing evidence for improved multiorgan function in the aged animal and deterioration in the young one. When parabiosis was sustained for three months, there was evidence of extended lifespan, marked reversal of epigenetic clocks, improved physical function, and rejuvenation by tissue omic signatures, which lasted months after the mice were detached from each other. The results have not yet been replicated, but another set of independent experiments refuted the benefit. Assuming the salutary and sustained effects hold up for parabiosis, although the results are intriguing, we're not going to surgically connect humans, so we need a more practical path, no less a hunt for the explanation of what is providing the benefit.

Systemic administration of young plasma (eight times over twenty-four days) to aged mice yielded improved cognition and memory, marked increase in new neurons (neurogenesis), and strengthened connections of hippocampus circuit neurons (known as synaptic plasticity), mimicking what was achieved via parabiosis. When half the plasma in old mice was replaced by a saline-albumin infusion, via a single thirty-minute procedure, there was replication of multiorgan benefits including cognition, less inflammation, reduced liver fibrosis,

and muscle repair. That raised the potential that rejuvenation of old mice was more of a function of diluting proteins in old blood. Other hints for what was driving the benefit came from the blood of exercised animals. Administering the plasma from exercised aged mice to sedentary counterparts improved cognition and hippocampus synaptic plasticity. A blood factor derived from the liver (Gpld1) was a mediator.

But the pro-youthful factor(s) in young mice blood has/have yet to be nailed down. Studies have raised many candidates, such as growth differentiation factor 11 (GDF11), osteocalcin, Klotho, platelet factor 4 (PF4), small extracellular vesicles, and a long list of others. When Klotho was given to nonhuman primates at low dose with a single injection, it enhanced memory and cognition. Regarding PF4, direct administration simulated the benefits of young plasma for rescuing cognition in aged mice and reducing brain inflammation. Proprietary plasma factors are being tested in clinical trials for Parkinson's disease with cognitive impairment and mild to moderate Alzheimer's disease.

The search for the youth factor has also been undertaken through the cerebrospinal fluid, since transferring young CSF to old mice provides similar benefits as plasma infusions. Fibroblast growth factor 17 (Fgf17) was sufficient on its own to achieve the effect. Most of these studies have been conducted in mice, but a more recent one taking young plasma from pigs infused to rats also showed similar benefits with reversal of epigenetic clocks.

Studies in people are scant. A small randomized trial of plasma donated by young people for nine patients with Alzheimer's backed up safety but without signs of benefit. Without knowing the specific factors or potential side effects, the strategy of diluting (by 60–70%) blood proteins through plasmapheresis (therapeutic plasma exchange, TPE) appears to be the safest way to test the hypothesis in three participants undergoing three rounds of TPE out to one year. This provided evidence of reduced inflammation, inflammaging, and less protein biomarkers linked to neurodegeneration and cancer.

Despite the lack of efficacy or safety in people, companies and clinics have been offering young plasma infusions and plasmapheresis for their putative antiaging effects. Ambrosia offered plasma from people aged sixteen to twenty-five years for $8,000 a liter. In 2019, the FDA issued a warning about young

donor plasma infusions that included language that "some patients are being preyed upon by unscrupulous actors." Nonetheless, there are still companies selling young plasma infusions, and TPE is still being offered by Next Health for $10,000, the Young Blood Institute, Maxwell Clinic, and many other companies. It's important to emphasize that besides the expense and lack of any proof for benefit, there are significant hazards of plasma infusions and plasmapheresis.

The young to old mice rejuvenation benefits extend beyond blood and cerebrospinal fluid to the gut microbiome, whereby fecal microbial transplants showed improvement in gut, eye, and brain function in aged mice, which all went the opposite direction with transplants from old to young mice.

Enough about old blood; it's time to move onto old, zombie senescent cells and how to get rid of them and avoid their deleterious impact. Back in 2015, my colleagues at Scripps Research published, in collaboration with Mayo Clinic researchers, a study of how well a combination of senolytics can kill senescent cells in mice. There is now abundant evidence of benefit for this intervention in mice. The first-generation senolytic drugs include the tyrosine kinase inhibitor dasatinib, an approved drug for treating leukemia, and naturally occurring flavonoid plant metabolites such as quercetin and fisetin. The senescent cells are resistant to cell death, apoptosis; to address this, another group of drugs, BCL-2 inhibitors like navitoclax and other pro-apoptotics, are in this group. Second-generation drugs include engineered (CAR-T) cells targeting senescent cells and drug or nanoparticle approaches to improve waste disposal of the senescent cells, such as glutaminase 1 (GLS1) or others targeting beta-galactosidase. Unlike the young blood story, there are many ongoing early phase (½) clinical trials and programs testing senolytics, mostly with the first-generation compounds and often in combination. There are ongoing trials in pulmonary fibrosis, kidney disease, Alzheimer's, osteoarthritis, sepsis, frailty, and diabetic macular edema. Early, small trials have indicated proof of clearing senescent cells with dasatinib and quercetin in diabetic kidney disease and idiopathic pulmonary fibrosis, but failed to provide clinical benefit in knee osteoarthritis or bone metabolism in postmenopausal women.

In a trial of diabetic macular edema for nineteen patients who failed conventional therapy, dose escalation of a pro-apoptotic drug (foselutoclax, a

BCL inhibitor, Unity Biotechnology) given directly into the eye promoted visual acuity, vascular repair, and favorable remodeling of the retina. In a study of five patients with mild Alzheimer's disease using oral (pills) dasatinib and quercetin for twelve weeks, eleven plasma-based and six cerebrospinal fluid–based biomarkers of cellular senescence improved. There was also evidence that some pro-inflammatory molecules were released, such as interleukin 6, which may indicate an unwanted inflammatory reaction to disintegrated senescent cells. In postmortem brains of patients with severe COVID, there was evidence of abundant senescent cells compared with age-matched controls. Different senolytics cleared the cells in organoid experiments. In two different animal models of COVID, senolytics cleared senescent cells and markedly reduced inflammation. Incorporating deep-learning AI has accelerated discovery of new potent senolytic small molecules.

The widely prescribed sodium-glucose cotransporter inhibitor (SGLT-2) drugs, like empagliflozin (Jardiance) or canagliflozin (Invokana), have been shown to reduce mortality in people with type 2 diabetes, and major outcomes in people with heart failure with preserved ejection fraction, but the mechanism for this benefit has been elusive. In the mouse model, SGLT-2 inhibitors eliminate senescent cells and improve survival, even when started at middle age. If this is verified in humans, it would be interesting to repurpose this class of drugs for promoting longevity.

A second-generation senolytic using engineered T cells (CAR-T) targeting the urokinase plasminogen receptor was tested in aged mice. Beyond clearing the senescent cells, a single injection provided lasting improvement of exercise capacity and metabolic function, including lower blood sugars. This work with CAR-T has been extended to rejuvenating the gut of aged mice. In a parallel way, gearing up natural killer T cells for removal of senescent cells, which worked in aged mice with lung fibrosis, is moving toward clinical assessment.

One missing link is a precise way to target senescent cells for these various elimination strategies. The problem, as touched on earlier, is that some senescent cells are doing good things in the body, and we currently don't have a selective approach to eliminate only the bad ones. It's just not that simple, as you might have been led to believe from the various mouse experiments I've reviewed.

That's where the Cellular Senescence Network (SenNet) can kick in to provide a panel of markers that may increase the efficacy of senolytics. It is still early in the human assessment of senolytics, but some promising early data suggest these may ultimately provide some benefit. An alternative route to clearing cells is revving up the waste removal from cells by promoting autophagy, through molecules like spermidine, urolithin A, or the function of mitochondria or lysosome subcellular organelles. It's a pathway that has panned out in small animal models but without clinical trial corroboration to date.

Is there a marker of old age more infamous than menopause? As the organ clock data indicated, estrogen replacement was associated with slower aging across many organs, especially the immune system, brain, liver, and arteries. We've long known that menopause is linked with an increase in age-related conditions, including heart disease, decreased bone health, and immune system dysfunction. As an alternative to the risks of hormone replacement, prolonging the lives of ovaries is getting attention. When an old mouse receives a transplanted ovary from a young mouse, her lifespan is expanded. Oviva Therapeutics is testing a hormone in mice and cats that expands ovarian functionality over time. A small randomized clinical trial is testing 5 milligrams of rapamycin weekly versus placebo for three months in women aged thirty-five to forty-five years who have evidence of menopausal transition and fulfill multiple hormone level inclusion criteria. The primary outcome is a measure of ovarian reserve, a surrogate for prolonging ovarian function. It's in the early phase of testing, but, as Renee Wegrzyn, director of Advanced Research Projects Agency for Health, aptly pointed out, "Ovaries are the only organ in humans that we just accept will fail one day." That passive acceptance is clearly getting a relook.

We must also turn to our good old friends, dogs. In a randomized trial of a proprietary senolytic with a NAD+ supplement (more on that later) in fifty-nine senior dogs testing low dose, full dose, or placebo, there was modest benefit of owner-reported cognition scores with the full dose. That's controversial, but while we're onto dogs, there's another intervention that could be groundbreaking for the regulatory side of antiaging.

The company is called Loyal Dogs and the proprietary drug (LOY-001) reduces insulin growth factor (IGF-1), which may account, at least in part, for

why small breeds of dogs live considerably longer. An injectable given every three months for dogs more than seven years of age and forty pounds reduced IGF-1 levels, and an ongoing placebo-controlled trial is underway in more than one thousand dogs for potential lifespan extension. Loyal Dogs has two other drugs (properties not disclosed) in pill form that will enter large randomized trial testing. While we know nothing about these drugs for efficacy or safety, the key point is that the FDA has given provisional acceptance of a drug for extending lifespan. That's why the announcement landed on the front page of the *New York Times* in late 2023.

The other drug that is being tested in dogs at some scale is a randomized trial of rapamycin for lifespan in the Dog Aging Project, an ambitious NIH-funded initiative that is tracking nearly fifty thousand pet dogs with a biobank of more than fourteen thousand tissue and blood samples, more than one thousand dog genomes sequenced, but its future funding is imperiled.

It's good that rapamycin is being tested in dogs for lifespan and health span extension since there are no such large-scale studies in humans. Similar to the effects of caloric restriction for decreasing protein synthesis and improving waste removal (autophagy), rapamycin and its derivatives (rapalogs), inhibitors of the mTOR gene that influence so many key cellular processes, are good *candidate* drugs for promoting health span. Of the twelve hallmarks of aging that we've been discussing, rapamycin, by inhibiting mTOR, has the potential to favorably modulate many of them.

Lifespan extension has ranged from 20 percent in worms to 60 percent in mice. Studies with rapalogs in aged individuals indicate some reduction of immunosenescence in response to vaccination or to viral infection with improved interferon-induced response. Paradoxically, the drug induces some immunosuppression with increased vulnerability to infections, an unnerving side effect. An exhaustive review of all the literature on rapamycin showed lack of evidence of an effect for many age-related issues, such as cognition, memory, muscle mass, or glucose regulation. But topical rapamycin did reduce biomarkers of aging in skin. Despite all the data that has accumulated for rapamycin and its derivatives, the optimal dose is unknown, and there are no meaningful ongoing clinical trials to test the hypothesis of impact on an age-related disease or the

aging process. Without such validation, and considering the known side-effect profile, there is no justification for its use at this juncture.

Metformin, on the other hand, with many overlapping biological effects on autophagy and mitochondria function, is being assessed in an ongoing trial of three thousand participants aged sixty-five to seventy-nine years. That study, however, is having difficulty getting fully funded and, even if successfully completed, is unlikely to be large enough to provide adequate evidence of efficacy. A systematic meta-analysis of 166 studies suggests this drug may be associated with a lower incidence of many types of cancer. But essentially all the data for this drug comes from observational, nonrandomized trials. Moreover, it has the especially counterproductive side effect of reducing the ability to build muscle mass with exercise in older adults.

Grouped here is also the nicotinamide adenine dinuclelotide (NAD+), produced by mitochondria and with a central role in energy metabolism. As one of the most common metabolites in our body, levels decrease with age, and more prominently among individuals with age-related diseases. Our cells are unable to take up NAD from our diet, but, ironically, the NAD precursors nicotinamide riboside (NR) and nicotine mononucleotide (NMN), marketed widely without clinical trial to show any benefit, are expected to reach over $1 billion in global sales in the next two years. A prime example of a market failing to guide us to the best product. NAD+ is also interconnected with the sirtuin pathway, a pathway once promoted as a critical lifespan expander (think of red wine's resveratrol), but proof of such a role has failed to be reproducible. The Sir/sirtuin family of proteins is derived from the words *silent information regulator*. From all the evidence thus far, "silent" is a better way to think of the role of the sirtuin family in modulating much less reversing the aging process.

Another mitochondria-targeted drug that has completed early clinical trials activates adenosine monophosphate-activated protein kinase (AMPK, Cambrian Bio, ATX-304), the enzyme viewed as "the guardian of metabolism" and a key means of restoring mitochondrial function. The jury is still out.

We began this part of our discussion of antiaging with immune-enhancing approaches, so it is fitting we conclude with anti-inflammatory drugs. These drugs are beginning to be assessed in clinical trials. The GLP-1 family of drugs

have been shown to improve various aging clocks in old mice at least as well as rapamycin, with particular impact on reversing the epigenetic clock of the hypothalamus. The ability to reduce the toll of age-related diseases affecting the heart and kidneys has already been shown, and there are encouraging data for potential disease-modifying benefit for Parkinson's and ongoing clinical trials in Alzheimer's disease. If it is proven beneficial for preventing or slowing the progression of neurodegenerative diseases, the suppression of brain inflammation by the GLP-1 drugs through the gut-brain axis, despite their lack of direct penetration across the blood-brain barrier, may turn out to be the fundamental mechanism. The company BioAge is assessing the combination of a GLP-1 drug and azelaprag (mimicking apelin, a muscle peptide that is reduced with aging) in a clinical trial for extending health span.

There are several other potent anti-inflammatory drugs that are candidates to modulate the aging process. Blocking the mitogen-activated protein (MAP) kinase (losmapimod) had a marked effect in skin of older adults for reducing inflammation while improving immune cell function. I've mentioned the promise of inhibiting the NLRP3 inflammasome. Another group of powerful inflammation blockers inhibit the cGAS-STING pathway (cyclic GMP-AMP synthase stimulator of interferon genesis), which has been implicated in inflammaging of the aged brain. Treatment with a cGAS-STING blocker in aged animal models markedly reduced inflammation in multiple tissues and restored innate immune cell function. Reversing fibrosis, the untoward organ scarring that can develop with aging, is being pursued in clinical trials with new drugs identified that also have strong anti-inflammatory action. Multiple other paths for reducing inflammation are being pursued, such as manipulating the gut microbiome.

The multiplicity and intensity of efforts underway to alter the human aging process, albeit still preliminary, are marvelous. Transforming aging isn't going to be easy or accomplished with a silver bullet, but in the last chapter we'll run through a path forward.

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I. The NLRP3 inflammasome protein complex abbreviation stands fornucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3.

# 13

# The Path Forward

Let's circle back to our nonagenarian friends from chapter 1: Mrs. L. R. and Mr. R. P., who exemplify two kinds of healthy aging. She appears to be a stroke of good luck; someone nature has gifted with unusually long health span. Nothing medical science can take much credit for. It doesn't even look like the genes she inherited had much to do with it.

Mr. R. P., on the other hand, had heart bypass surgery and later some stents along with extensive cardiovascular care. He had a shoulder replacement accompanied by a heart attack, and, with prompt medical care, he survived COVID.

While I did provide Mrs. L. R. some modest professional attention, and it is fair to say Mr. R. P. received critical state-of-the-art health care in his tenth decade, neither of them had any kind of whole-body aging halt or reverse. Overriding the aging process poses profound challenges. It's hard to prove in people. The gold standard of a large-scale randomized trial, with one group of participants receiving the intervention and the other a placebo control, on a blinded basis (double, for both the researchers and participants), would require many years of follow-up, likely a decade or more. None of the many companies that are developing interventions to slow aging are going to invest in such a clinical trial. So we are left with having to use surrogate endpoints like epigenetic or proteomic clocks, which do not represent clinical outcomes.

As I have noted, many of the potential ways we must alter the pace of aging carry a serious risk of cancer. That's because the biologic hallmarks of aging are remarkably convergent with the hallmarks of cancer. Aging is characterized by genome and epigenome instability, shortened telomeres, loss of protein regulation (proteostasis), dysfunctional mitochondria, old senescent cells and exhausted stem cells, impaired cellular waste disposal, chronic inflammation, diminished immune system responsiveness, and an imbalance of the gut microbiome. Most, if not all, of these features of aging promote cancer. As we've seen, epigenetic reprogramming, lengthening of telomeres, and engineered T cells all have a potential liability for inducing cancer or its progression. Take senescent cells in our body as an example. They can no longer divide, so this is a way to protect against cancer. But the trade-off is that these cells are dysfunctional and secrete pro-inflammatory proteins. The burden of proof is that "eradicating" old cells through senolytics is advantageous compared to our natural way of shutting down these cells. Senolytics don't simply vaporize the senescent cells; they may leak and become deleterious in the process. It's hard to precisely target them, so healthy cells may be roped in. Or revving up our waste disposal system (autophagy) could provide a distinct benefit for any cancer cells that exist in the body. Improving mitochondrial function could also lead to a gain of metabolic fitness for cancer cells. Moreover, the various strategies for antiaging assume a homogeneous effect on all cells. If we do partial epigenetic programming, there's no assurance that will occur evenly; some cells might be fully reprogrammed and potentiate the risk of cancer. Likewise, what if we rejuvenate our immune system, reversing immunosenescence, but set off self-directed autoimmunity?

One of the other obstacles that holds back a drug or intervention to promote lifespan is that aging is not considered a disease by regulatory agencies. You will recall that obesity was not considered a disease until the GLP-1 drugs emerged, which radically altered our perception of this condition. The same might eventually occur with aging, but it's different because aging is universal, whereas obesity is not. Both are linked with a wide range of diseases. We'll have to see what effect that evidence, while currently lacking, has in the future. It is likely that preventing a specific age-related disease will comprise the evidence someday, even though that does not equate with body-wide slowing of aging.

We have little data to support that slowing the aging process leads to a "compression of morbidity," the concept initially proposed by James Fries in the early 1980s. It can be likened to the 1858 poem by Oliver Wendell Holmes about the horse carriage, "The Deacon's Masterpiece or the Wonderful One-Hoss Shay":

*Have you heard of the wonderful one-hoss shay,*
*That was built in such a logical way*
*It ran a hundred years to a day,*
*And then, all of a sudden it*
. . . . . .
*How it went to pieces all at once,*
*All at once, and nothing first,*
*Just as bubbles do when they burst.*

Fries proposed that over time we are moving toward an "ideal survivorship curve," meaning that we extend healthy aging and there's minimal time for chronic illness (fig. 13.1, left panel), as if a person suddenly falls off the cliff or dies with intact health in their sleep. This same concept was also popularized by Peter Attia in his book *Outlive* as "Medicine 3.0" (fig. 13.1, right panel).

Ray Kurzweil, who believes that the first person who will live to one thousand years has been born, is a big proponent of "longevity escape velocity," as he wrote about in the *Economist* and his book *The Singularity Is Nearer*. The idea, introduced a couple of decades ago, is that eventually medical advances will outpace life expectancy. Kurzweil believes that annual increases in life expectancy will reach twelve months between 2029 and 2035, "at which point ageing [for people] will not increase their annual chance of dying." Things just don't move at such velocity in medicine. But, again, expanding longevity does not equate to living longer with fewer years of chronic illness.

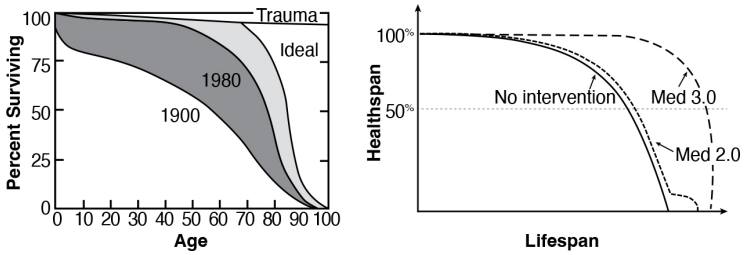



***Figure 13.1.*** Theoretical compression of aging. Ideal survivorship curve proposed by James Fries (left panel) and Medicine 3.0 curve proposed by Peter Attia (right panel). Adapted from James Fries, "The compression of morbidity," *Annals of the Academy of Medicine of Singapore* 12, no. 3 (July 1983): 358–67 (left panel); and Peter Attia, *Outlive: The Science and Art of Longevity* (New York: Harmony, 2024) (right panel). Description 64

While it would be ideal for people who made it healthy well into their eighties or nineties not to have to suffer dementia, cancer, cardiovascular disease, or frailty for many years, it's hard to find any support for this concept. Perhaps the only finding backing this comes from the New England Centenarian Study on the rarefied group of supercentenarians (age 110 and older) who were healthy throughout their lives and did not exhibit disability in their last years. There's no evidence yet for safely and effectively slowing the aging process in human beings. Until proven otherwise, we should assume that expanding health span or slowing aging simply defers morbidity rather than compresses it.

Notwithstanding these difficulties, I do believe we will ultimately see body-wide modulation of aging. We have many different promising shots on goal, huge resources being expended, and acceleration of drug discovery efforts with AI. We have seen with the GLP-1 drugs how, despite decades of blatant failures, it was ultimately possible to tackle one of the most profound medical challenges of all—obesity. And it would not be at all surprising that a derivative of that drug class pays dividends for extending health span. That's in the cards for the future, not now.

Here is where the excitement is well founded and near term. We are going to see major progress in prevention of age-related diseases and preserving organ-specific health. We're now entering a golden time when we'll be doing that on an ever frequent and impactful basis. That's because most age-related diseases have a long warm-up period of at least one to two decades before symptoms manifest. With biomarkers, polygenic risk scores, proteins, genome sequencing, imaging,

and multimodal AI, we are gaining unprecedented power to identify high-risk status at the individual level. Not just identify high risk but do so early in a person's life and predict the timing of when the condition may begin to manifest. AI appears to be capable of reliably predicting tipping points before they happen in complex systems, and the human body surely qualifies as a complex system.

This precision medical forecasting allows for intensive lifestyle+ factors to be put in place for the individual, along with tight surveillance with biomarkers such as protein organ clocks, cell-free tumor DNA in the plasma, inflammation, gut microbiome constituents, or appropriate imaging aligned with the condition of concern. This strategy is new and awaits validation, but it is a less ambitious, more realistic and attainable one than changing body-wide aging.

In the meantime, there's no hard line between modulating whole-body aging and targeting a specific organ's aging. We know GLP-1 drugs can help prevent diabetes and heart failure in people with obesity, among other organ effects. You could interpret that as having multiorgan benefit. Maybe this family of drugs favorably affects the aging process throughout the body. We'll eventually find out. Likewise, a potent anti-inflammatory drug or partial epigenetic programming could be used to target an organ but might wind up slowing the aging process throughout the body. On the other hand, a body-wide directed aging intervention may only slow the pace of aging in one organ. For now, organ-specific interventions can be considered as building on what we know, de-risking the safety concerns for being overly aggressive. If we could prevent the major diseases that are age-related, that would be a monumental achievement. I'm convinced that is now possible.

It's the era of digital biology. As Jensen Huang, CEO of Nvidia, put it: "For the first time in human history, biology has the opportunity to be engineering, not science." I would say both. How we can engineer healthy aging—prevention of age-related diseases—is an outgrowth of accelerated life science output in recent years. The exponential growth we have seen with generative AI will be paralleled by the same in digital biology (fig. 13.2). Evolutionary Scale, formed by scientists from Meta, created a protein language model with 2.7 billion protein sequences, structure, and functions; we're on the path to making biology programmable. The dizzying pace of innovations that span the gamut from

genome editing, controlling our (tolerogenic) immune response, identifying critical biomarkers for tracking early cancer and neurodegenerative diseases, engineering cells, and delivering biologics via mRNA and nanoparticles is all taking hold at one time. Collectively, we are witnessing an incomparable time in life science.

The target to prevent or markedly delay age-related diseases that I find most intriguing, as will probably be obvious by now, is our immune system. There's a pervasive belief that we're either immunocompetent or immunocompromised. That's wrong! We desperately need a way to systematically assess our immune system. By conducting an "immunome," we will be able to predict a person's response to infections, vulnerability to cancer or its spread, and propensity for developing cardiovascular and neurodegenerative diseases. Today, we do little to characterize a person's immune system's status beyond a conventional blood test. Although this tells us the number of white blood cells (neutrophils) and lymphocytes, it is grossly insufficient. Even the neutrophil to lymphocyte ratio has some negative prognostic information for lifespan when high, but we ignore that. A decade ago, we saw how our exposure to more than one thousand virus strains and timing could be accurately detected through antibodies from a drop of blood for the low cost of $25. That would tell us about our exposure to cytomegalovirus, a key exposure that tracks with older immunotypes, associated with an aging immune system. But this test is still not clinically available.

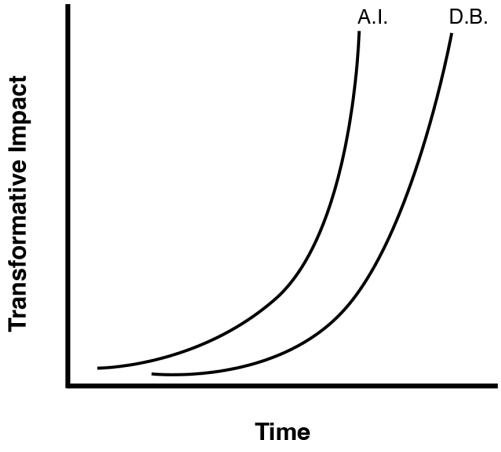


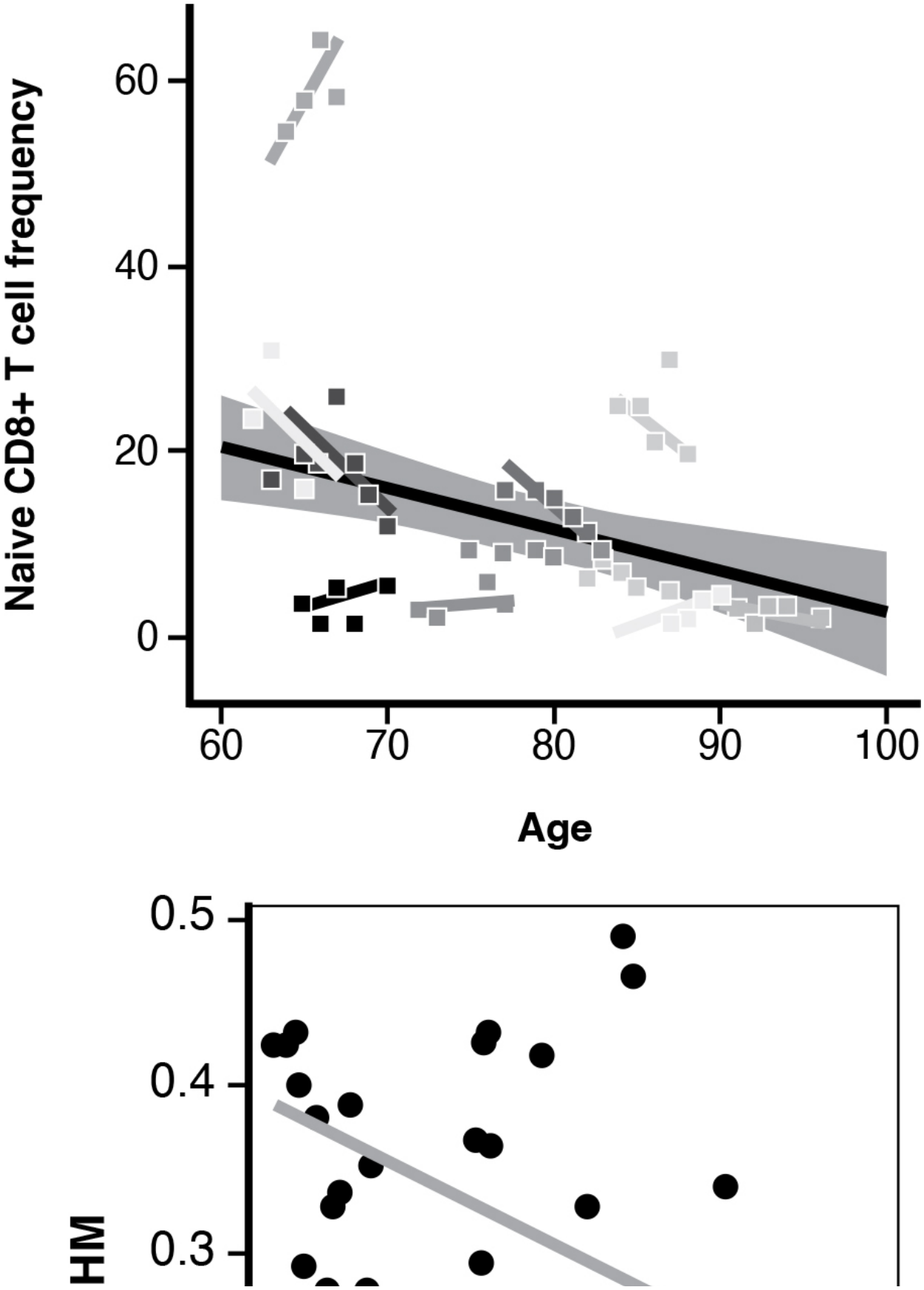

***Figure 13.2.*** Exponential curves of transformative impact over time for generative AI and digital biology
Description 65

What if we could periodically grade an individual's immune system? One of the most provocative studies in this regard was published several years ago, tracking 135 healthy individuals over nine years, each year assessing a participant's immune system for their immune cell profile (T, B, NK, and subtypes), gene expression, and cytokine production. One finding was the decline in CD8+ cytotoxic T cells with age, which was highly variable from one individual to another (fig. 13.3). The immunologic age clock from this study was superior to epigenetic clocks for predicting mortality.

A second composite of immune cells and their proteins (cytokines and chemokines) is iAge score. Its increase correlates with chronological age, frailty, cognitive decline, and the number of coexisting conditions. Of note, centenarians had a low iAge score compared with people aged fifty to seventy-nine years old. A third immunome based on CD4+ helper T cells, CD8+ cytotoxic T cells, and gene expression predicted the best survival over a decade of follow-up for the group with markers of high immunocompetence and low inflammation.

More recently, a stab at another immunome called the immune health metric was developed from blood proteins, RNA sequencing, immune cells, and clinical data, integrated with multimodal AI. It can differentiate marked aging in clinically heathy people, track the immune response to diseases, and predict the age-dependent antibody response to vaccines. Like the prior study, there is marked heterogeneity for the metric from one individual to another (fig. 13.3).

Ben Larman, a scientist who runs the Laboratory of Precision Immunology at Johns Hopkins, started a company called Infinity Bio that, amazingly, can take a small amount of blood (a drop) on filter paper and through extensive panels of DNA-barcoded proteins and protein fragments determine a person's exposure to over five hundred viruses via their antibodies. In the same assay, the new technology can detect hundreds of autoimmune antibodies. The test, which has a ring of the Theranos fraud debacle except that it is real, is currently only available for research projects since the company is taking a rigorous stepwise approach—which makes sense given the promises of Theranos's fake tests. Hopefully Infinity Bio's test will become commercially available for clinicians and consumers in the next couple of years. For a preview, my results for this test are presented in figure 13.4.


Naive CD8+ T cell frequency
60
40
20
0
60
70
80
90
100
Age
0.5
0.4
0.3
HM

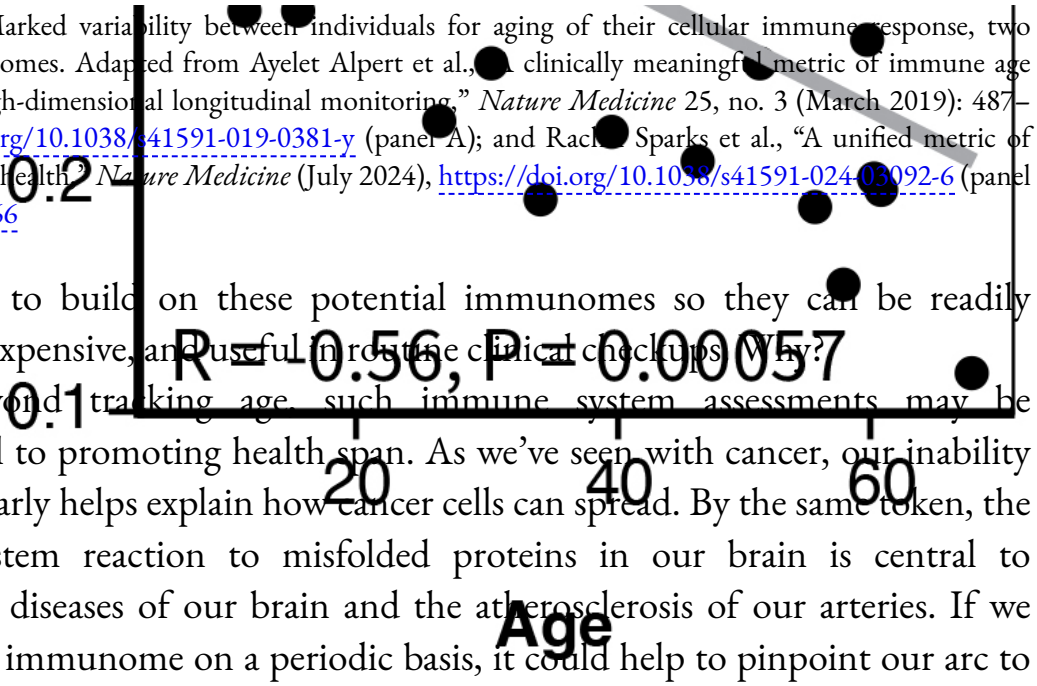
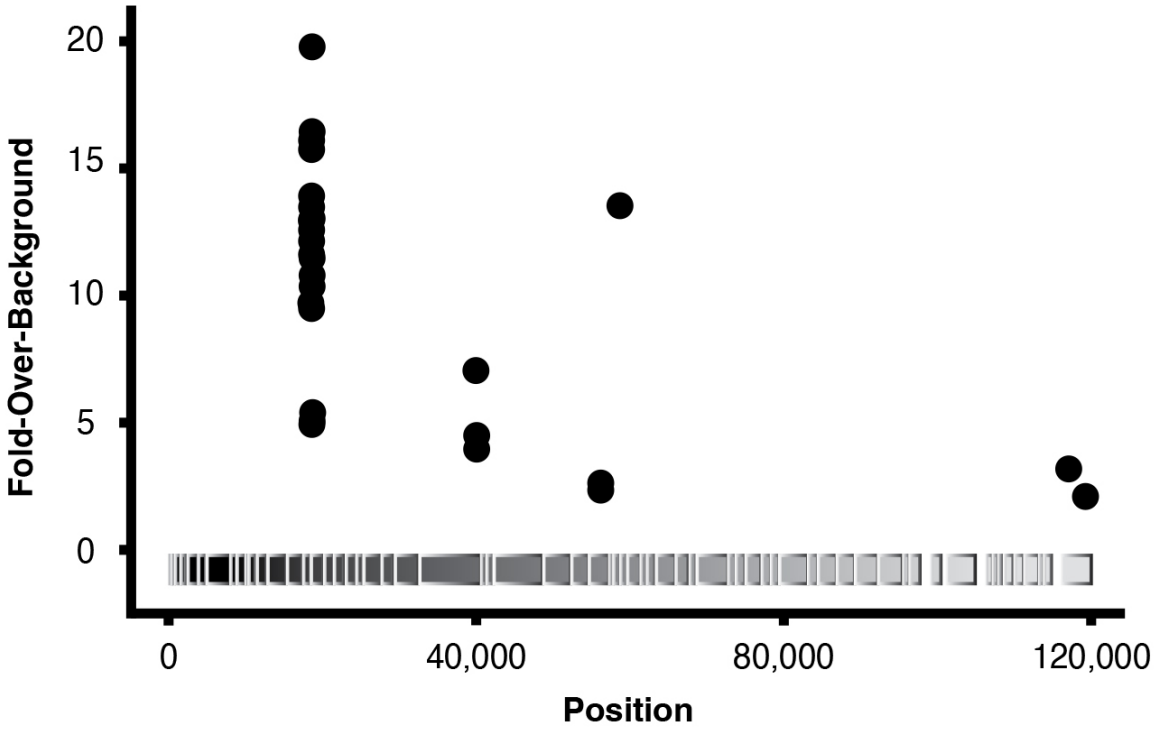



***Figure 13.3.*** Marked variability between individuals for aging of their cellular immune response, two different immunomes. Adapted from Ayelet Alpert et al., "A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring," *Nature Medicine* 25, no. 3 (March 2019): 487–95, https://doi.org/10.1038/s41591-019-0381-y (panel A); and Rachel Sparks et al., "A unified metric of human immune health," *Nature Medicine* (July 2024), https://doi.org/10.1038/s41591-024-03092-6 (panel B). Description 66

We need to build on these potential immunomes so they can be readily available, inexpensive, and useful in routine clinical checkups. Why?

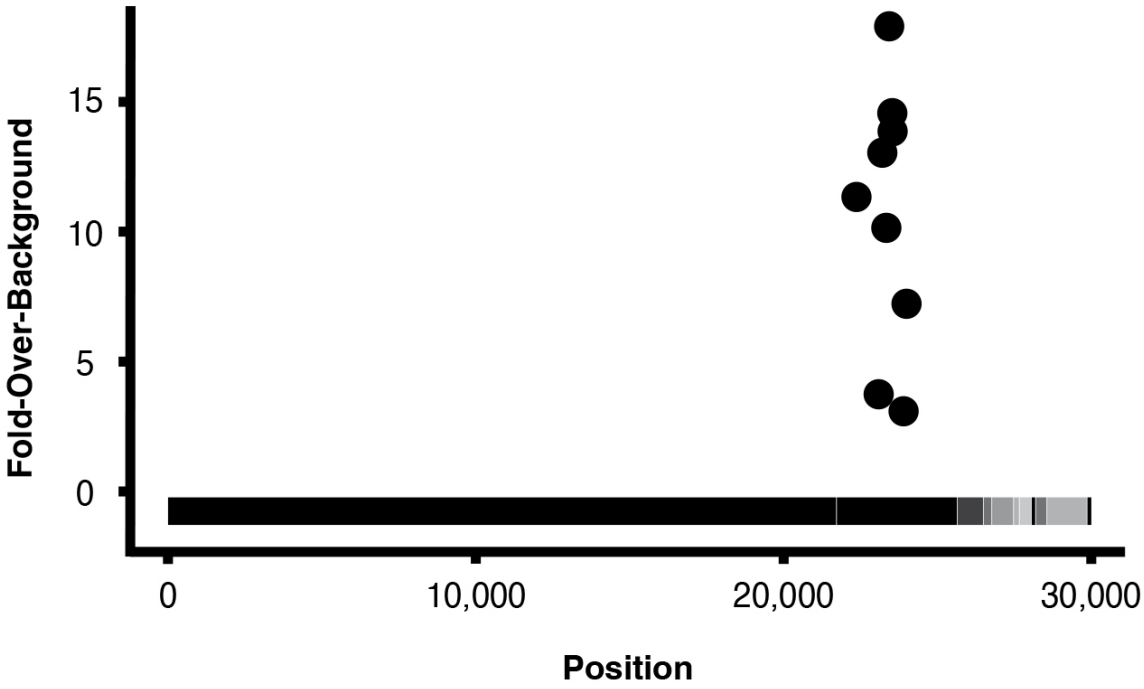
Well beyond tracking age, such immune system assessments may be fundamental to promoting health span. As we've seen with cancer, our inability to quash it early helps explain how cancer cells can spread. By the same token, the immune system reaction to misfolded proteins in our brain is central to degenerative diseases of our brain and the atherosclerosis of our arteries. If we could get an immunome on a periodic basis, it could help to pinpoint our arc to immunosenescence and guide our decisions about potential interventions such as a vaccine or some other treatment (senolytic, gut microbiome manipulation, or partial epigenetic reprogramming) to rejuvenate our immune system. That would enhance our defense against many age-related diseases. The flip side is that supercharging our immune system could prove to be unsafe, if it results in a pro-inflammatory state from stimulating release of cytokines or chemokines or fosters autoimmunity. Sure, it may be a Goldilocks strategy with the need for just the right level of immune response in the right person at the right time, but that is the approach nature seems to be demanding.

Until now, our medical approach has been reactive, with secondary prevention at best. A patient has a heart attack; we put them on statins and aspirin to prevent another event. For a diabetic, we treat blood sugar and lower the hemoglobin A1c. While there has been steady improvement in cancer treatments and less reliance on classic chemotherapy, most patients are not getting diagnosed early and the prognosis has extended survival a matter of months for many of the most successful new treatments. We're in a reactive rut. But now we have the chance to fulfill the ultimate medical dream of primary prevention. It's within our reach.




| Virus Exposure Aggregate Reactivity Score | Topol | Control |
|---|---|---|
| Rhinovirus A | 16 | 20 |
| Epstein-Barr virus | 10 | 22 |
| Herpes Simplex virus | 10 | 1.2 |
| Respiratory syncytial virus | 8 | 13 |
| Rhinovirus B | 7.9 | 11 |
| Varicella zoster virus | 6.4 | 1.8 |
| Influenza C virus | 5.9 | 0 |
| SARS-CoV-2 virus | 5.5 | 3.2 |
| Avian influenza (H7N9) virus | 4.7 | 0 |
| Norwalk virus | 3.9 | 6.9 |

***Figure 13.4.*** Legend on the following page. Description 67




| Autoantibody to proteins or peptides | Topol | Control |
|---|---|---|
| ZTB5 (zinc finger and BTB domain-containing protein 5) | 50.3 | 1 |
| FNDC4 (fibronectin type III domain-containing protein 4) | 27.6 | 1 |
| ARMC5 (armadillo repeat-containing protein 5) | 23.23 | 1 |
| UBE22 (ubiquitin-conjugating enzyme E2) | 20.29 | 6.88 |
| CDH12 (cadherin-12) | 11.67 | 1 |
| ARRB1 (beta-arrestin-1) | 10.84 | 1 |
| CASP8 (Caspase 8) | 9.22 | 1 |
| PHLDA1 (apoptosis-associated nuclear protein) | 8.21 | 1 |
| USP48 (Ubiquitin carboxyl-terminal hydrolase 48) | 7.63 | 1 |
| METTL3 (Methyltransferase like 3) | 7.22 | 1 |

***Figure 13.4.*** My results from the Infinity Bio assays. Prior page is my antibody response to the chickenpox proteome, revealing the specific regions strongly recognized by my immune system. This page shows my response to SARS-CoV-2, which is focused on the spike protein, the component included in the vaccines. My antibodies do not recognize other regions of the virus. The table (page 327) shows my aggregated virus

reactivity scores compared with control for selected viruses of the 529 species covered in the test (~2.4 million viral proteins represented by overlapping peptides). I have no antibodies to cytomegalovirus. Table above shows some of the human proteins that my autoantibodies recognize (from 27,703 genes covered in the test). Description 68

Take Mr. R. P.'s case. He could have had a multilayered assessment at an early age for risk of cardiovascular disease (as depicted in fig. 5.8) and received aggressive primary preventive strategies, including statins and lifestyle+ factors, along with active surveillance, so that he would have never required coronary artery bypass surgery or stenting, nor would he have suffered a heart attack. We now have the blueprint, the five dimensions, and extraordinary potential to suppress all the major age-related diseases.

Before my optimism gets too far out front, let me reiterate the two factors that remain the most formidable obstacles. One is our profound health inequity. This has positioned people who are underrepresented or with low socioeconomic status at the highest risk, not only for their lifestyle+ factors such as poor nutrition or air pollution but also for their access to all the innovations discussed in this book. If expanding health span turns out to be only for the rich and privileged, then it can be considered an abject failure. Just as we are now in a unique position for digital biology to deliver exponentially better public health results, we must address equitable access to health span expanders. The prime example today is the GLP-1 family of drugs, which are not affordable for the vast majority of people who need and would benefit from them the most. Public-private partnerships to find a solution that provides these drugs for no or nominal charge could be a template for many of the other digital biology innovations that become available in the future. Our lack of a universal health system in the United States, the singular outlier of rich countries in the world, puts us at an unnecessary disadvantage for promoting health span expansion at the population level. Health care should be considered a civil right. Our inability to address this albatross of unfairness holds us back.

The second great obstacle is about chasing our own tails. How can we simultaneously be hyper-innovative for extending health span but ignore all the things that are shortening it? Little to nothing has been done to address pervasive and worsening air pollution, micro and nanoplastics, the toxic PFAS forever

chemicals, intake of ultra-processed foods, and so many of the components that I have included in the updated lifestyle+ category, in the air we breathe and the water we drink. The unabated mis- and disinformation anti-science movement, centered around conspiracy theories spreading in social media and digital media of all kinds, threatens the acceptance and use of momentous discoveries. Anti-science is a threat that can only be addressed through a restoration of trust in biomedical expertise.

As we move forward, we will inevitably see suppression of age-related diseases that for many is unimaginable right now. Squashing age-related diseases is such an exciting prospect, but it reminds me of what we saw in the first year of the COVID pandemic. There was no vaccine, and deaths were rapidly mounting throughout the world, especially among older adults. Those who could hang in to get vaccinated achieved a far greater immune protection from a safe exposure to the spike protein and substantially enhanced survival. Now the analogy is that we need to maintain our healthy aging, predominantly by focusing on lifestyle+ means, so that we can get to the next phase of digital biology deliverables.

It will take years to accomplish the far bolder objective of slowing the aging process itself. But when we do, Wellderly folks, super agers like Mrs. L. R. and Mr. R. P., will seem like an ordinarily vibrant group of people making valuable contributions to society. I recently turned seventy, and I am hopeful that you and I will be among them.

# *Acknowledgments*

**W**ho wouldn't want to have a very long health span, with more years of life free of any significant disease? The surge of advances in life science and medical research is now pointing a way forward, but at the same time there is no shortage of miscues, lack of evidence, and unrealistic expectations.

As mentioned in the introduction, my research group and I have had a long interest in health span, as reflected by our Wellderly initiative of whole genome sequencing a large cohort of individuals aged eighty-five years and older without chronic illness. Rather than cracking the genes that account for this desirable trait, that work mainly taught us that it's far more complex. So how are we going to learn the underpinnings of a long, healthy life?

That big question prompted my work for this book, necessitating an in-depth review of all the recent and remarkable progress that spans many biomedical disciplines. It proved to be the most ambitious project that I've yet undertaken. I was fortunate to be able to call on many subject experts to review the chapters, and want to express my deep gratitude to Charles Swanton, Tony Wyss-Coray, Shane Crotty, Fyodor Urnov, Juan Carlos Izpisua Belmonte, Kristian Andersen, John Torous, and Paddy Barrett for their highly constructive input.

My colleagues at Scripps Research provided invaluable support. In particular, Michelle Miller was instrumental in culling all of the 1,800 citations together, a herculean task, and Faith Hark, a life science graphics artist, created more than seventy figures. Of immense help were the extended *Ground Truths* podcast conversations that I've had with leading researchers, including Venki Ramakrishnan, Daniel Drucker, Geoffrey Hinton, Steve Horvath, Pradeep Natarajan, Euan Ashley, Jennifer Doudna, Tom Czech, Coleen Murphy, Jim

Collins, Katalin Kariko, David Liu, James Zou, Patrick Hsu, Chris Van Tulleken, John Halamka, and Lotte Bjerre Knudsen. I'm also indebted to my Scripps Research co-faculty members, with whom I had frequent discussions, and my president and CEO, Peter Schultz, who has fostered an environment that maximizes such collaborative and creative effort.

After writing three books, this was the first time I had the distinct opportunity to work with the impressive team at Simon & Schuster. My primary editor, Stephen Morrow, provided instrumental input for the book, making it more accessible to general readers. He greatly inspired me with his unwavering support and enthusiasm for the content. I also had the privilege of working with Jennie Miller and with Priscilla Painton, editor in chief, whose editing contributions were important and deeply appreciated. It's a major challenge to break down complex science to language that's easy to understand for all readers, irrespective of their background. There's no question in my mind that collectively their wisdom and input helped favorably shape the final product. I've had the pleasure of working with my literary agent, Katinka Brockman, for well over a decade. I still remember our first conversation in 2008 when I told her I wanted to write three books and she replied, really? After I reached that goal six years ago, I never thought there was another one left in me.

Ironically, I have to thank the COVID pandemic. In its early days in 2020, I realized that we were being exposed to toxicity and danger beyond the virus. At a time when so many lives were on the line, when we faced an existential crisis of the worst pandemic in over a century, we were being fed blatant misinformation, some of it even from our own government leadership and public health agencies. Despite lack of any formal background in infectious diseases or epidemiology, I shifted my attention and efforts to share what I could learn. I started posting everything I read and analyzed each day on Twitter and other social media, wrote op-eds in various newspapers and in the *Ground Truths* newsletters on Substack, and did what I could to be regarded as a trusted source of information. After nearly four decades as a cardiologist, I never realized that I might become a COVID-ologist. But what I learned from that experience is the value of doing this work for the large proportion of people who were confused about what was happening, such as how to avoid infection, the role of vaccines and boosters, and

the sequelae of Long COVID. That also spawned our team's original research efforts to understand the many dimensions of this new illness, such as infections without symptoms, genomic mutations and evolution of the virus, and development of universal vaccines.

Had it not been for that experience with COVID, I likely would not have written this book. There are many parallels with the field of longevity, with recommendations for taking powerful drugs like rapamycin or getting total body MRI scans, without the requisite evidence that the benefit exceeds the harm. Or false expectancies that we will live to one hundred and suddenly "fall off the cliff," without experiencing any serious chronic illness. All of this prompted me to try and set the record straight, review everything relevant that has been published, and put it into context for now and the future. I may have made mistakes in interpreting some of the large body of data, but I've done the best I could to cite it so you can review it yourself and maybe come to a different conclusion.

That brings me to the important issue of bias. My financial disclosures of potential conflict of interest are that I previously served on the Board of Directors of Dexcom, a glucose sensor company, and currently serve on the Science Advisory Boards of Tempus, Danaher, Abridge and Pheno.ai. Exposure to the technologies and research initiatives of these companies has helped formulate my perspective. I have no relationship with any company working on longevity, or any biotech or pharmaceutical company. The Scripps Research Translational Institute that I founded in 2006 receives extensive funding from the National Institutes of Health.

I plead guilty to being a techno-optimist, as you may know from my prior books and writings, but I always try to find the balance and have learned that it takes much longer to see the putative benefits of technology rigorously validated and properly implemented than I've ever anticipated.

My favorite part of each workweek continues to be seeing patients. It is from them I learn what are our big unmet needs in medicine and the opportunities going forward. I'm especially indebted to two of my patients, whom I've described in this book, who were willing for me to tell their stories, which will hopefully help you understand the key differences between altering the body-

wide aging process and preventing, or markedly delaying, age-related diseases. For the latter, I am especially sanguine that we can and will be making big strides of progress in the near term.

Finally, I want to thank Susan, my wife, with our forty-sixth wedding anniversary on the day of this book's publication! Throughout our lives together, she has provided steadfast support for what I feel the need to pursue, all too often over-consuming. Hopefully, some of the content here will not only help you, but us, in having more healthy years together.

La Jolla, California

# *About the Author*

© ERIC TOPOL

ERIC TOPOL, MD, is the executive vice president and a professor of molecular medicine at Scripps Research, the largest nonprofit biomedical institute in the United States. He is also founder and director of the Scripps Research Translational Institute and a practicing cardiologist. He is one of the top ten most cited researchers in medicine, known for his groundbreaking studies on AI in medicine, genomics, and digitized clinical trials. He was named to the *Time*100 Health list of the most influential people in health in 2024. Dr. Topol writes the Substack newsletter *Ground Truths* and is the author of *The Creative Destruction of Medicine, The Patient Will See You Now,* and *Deep Medicine.*

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*Notes*

# PART I THE AGE OF HEALTH SPAN

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# PART III GREAT IMPLICATIONS

## 8. Curing Rare Diseases

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## 9. Controlling Our Immune System

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## 10. Defeating Infectious Agents

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# PART IV THINKING AHEAD

## 12. Changing Aging

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First Simon & Schuster hardcover edition May 2025

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*Interior design by Ruth Lee-Mui*
*Jacket design by Pete Garceau*

Library of Congress Cataloging-in-Publication Data has been applied for.

ISBN 978-1-6680-6766-6
ISBN 978-1-6680-6768-0 (ebook)

# *IMAGE DESCRIPTIONS*

Description 1: Each field is labeled along the outer edge of the circle, with curved bands inside the circle connecting the fields. The bands vary in thickness. BACK.

Description 2: Five arrows extend downward from this title, each pointing to a specific health condition. From left to right, the conditions listed are: "Cardiometabolic Diseases," "Cancer," "Neurodegenerative Diseases," "Autoimmune Conditions," and "Accelerated Aging." BACK.

Description 3: The vertical plot shows body weight change in kilograms ranging from negative 1.5 to 1.5 in increments of 0.5. The horizontal plot represents days on the diet, ranging from 0 to 14 in increments of 2. Two lines are plotted with error bars: a line for the ultra-processed diet and one for the unprocessed diet. The ultra-processed line begins at 0 kilograms on day 0 and rises steadily to approximately 1.2 kilograms by day 14. The black line also starts at 0 kilograms on day 0 but declines steadily to approximately negative 1.2 kilograms by day 14. BACK.

Description 4: The vertical plot shows the change in total mortality percentage, ranging from negative 30 percent to 20 percent. The horizontal plot shows the increment of energy from a specific type of fat, ranging from 0 to 5 percent. Four lines represent different fat types. The trans fat line rises steeply from 0 percent change to approximately 20 percent increase. The saturated fat line also increases, but gradually, reaching about 10 percent. The monounsaturated fat line declines slightly to around negative 10 percent. The polyunsaturated fat line declines most sharply, reaching about negative 25 percent. BACK.

Description 5: The vertical plot for both plots displays the odds ratio on a logarithmic scale ranging from 1 to 1000, while the horizontal plot represents alcohol consumption in drinks per week, ranging from 0 to 35. Plot A, titled "Hypertension," illustrates a curve that begins near an odds ratio of 1 at 0 drinks per week and rises steadily, with a sharper incline occurring after 14 drinks per week. A shaded area around the curve represents the 95 percent confidence interval, which broadens with higher alcohol consumption. Plot B, titled "Coronary Artery Disease," reveals a similar positive trend, with the odds ratio increasing as alcohol intake rises. However, the slope is less steep than in Plot A, though uncertainty similarly increases. BACK.

Description 6: The first scatter plot shows the relative risk of mortality on the horizontal plot and the average relative environmental impact on a logarithmic vertical plot. Red meats appear in the upper-right quadrant, while vegetables, fruits, whole grains, and nuts are in the lower-left. The second scatter plot shows the percent change in mortality risk on the horizontal plot (from minus 30 to 40) and environmental impact on the vertical plot (0 to 80). Processed meats show increased risk and impact; plant foods show reductions in both. The bar chart compares dietary patterns with greenhouse gas emissions per person per year. The average diet exceeds 2,000 kilograms of carbon dioxide equivalent; the vegan diet is under 500. BACK.

Description 7: Panel A, titled "Current Dogma (curvilinear)," shows a plot with health risk on the vertical plot and exercise training volume on the horizontal plot. The curve starts at the top left labeled "No exercise (highest risk)," then slopes steeply downward and levels off. Panel B, titled "Alternative Hypothesis (reversed J-shaped)," uses the same axes. The curve again starts at "No exercise (highest risk)" and declines steeply to a point labeled "Most benefit of exercise" at moderate levels. Beyond this, the curve rises slightly, labeled "Partial loss of health benefits." BACK.

Description 8: The vertical plot shows relative risk (RR) ranging from 0.6 to 2.0 in increments of 0.2, with a horizontal dashed line at 1.0. The horizontal plot

shows weekly resistance training duration from 0 to 200 minutes in increments of 50. A solid line represents the estimated RR, and two dashed lines indicate confidence intervals. The curve starts at about RR 1.0 at 0 minutes, decreases to a minimum RR of approximately 0.8 at 50 minutes per week, then rises again, crossing the baseline at around 100 minutes. The curve continues to climb, reaching approximately RR 1.5 at 200 minutes. The confidence intervals broaden with increasing duration. BACK.

Description 9: The regional table lists fat percentage, total mass, fat, lean tissue, and bone mineral content for arms, legs, trunk, android, and gynoid areas. Total body composition shows 23.9 percent fat, 180.7 pounds total mass, with 41.6 pounds fat, 132.1 pounds lean, and 7.0 pounds bone mineral content. The bone density section reports a T-score of 0.0 (normal) and a Z-score of 0.3 (50 to 69th percentile). A reference table explains diagnostic thresholds. Two scans are included, one of the skeletal structure and one color-coded image highlighting fat, lean, and bone tissue distribution across the body. BACK.

Description 10: The vertical plot shows the hazard ratio ranging from 0.4 to 1.0 with a 95 percent confidence interval. The horizontal plot represents handgrip strength in kilograms, ranging from 15 to 50 in increments of 5. A solid line represents the hazard ratio, which starts at 1.0 for 15 kilograms and declines steadily, reaching approximately 0.5 at 50 kilograms. Two dotted lines represent the 95 percent confidence interval, which broadens toward the lower and higher ends of the strength range, indicating increased uncertainty. Small vertical tick marks are distributed along the horizontal plot, representing individual data points for handgrip strength. BACK.

Description 11: The vertical plot in each plot shows adjusted hazard ratios (HR) from 0.5 to 3.0, with shaded 95 percent confidence intervals. The horizontal plot shows the average SRI (Social Relationship Index) from 40 to 70. In “All-Cause Mortality,” HR drops from 2.0 at SRI 40 to 1.0 at SRI 60. In “Cardiovascular Mortality,” HR falls more sharply from 2.0 to 1.0 by SRI 60, with a slight plateau. In “Cancer Mortality,” HR declines gradually from 1.5 at

SRI 40 to 1.0 at SRI 60, then trends slightly downward. Confidence intervals widen at both ends of the SRI range. All three plots show an inverse relationship between SRI and mortality and report statistical significance with p less than 0.001. BACK.

Description 12: In both plots, the vertical plot represents the outcome measures. In the left plot titled "Cognition," the vertical plot ranges from 0.4 to 0.8. In the right plot titled "Mental Health," the vertical plot ranges from 0.25 to 0.55. The horizontal plot for both plots represents sleep duration in hours, ranging from 1 to 10. In the cognition plot, the U-shaped curve starts at approximately 0.8 for 1 hour of sleep, declines to around 0.4 at 7 hours, and increases to about 0.6 at 10 hours. In the mental health plot, a dashed line shows an inverted U-shaped curve, beginning near 0.25 at 1 hour, peaking at 0.55 at 7 hours, and decreasing to 0.4 by 10 hours. BACK.

Description 13: The human silhouette inhales polluted air, with arrows pointing to body systems affected by pollution. The immune system shows increased Th1 cells, decreased Th2 and Treg cells, and enhanced bone marrow mobilization. Adipose tissue shows increased M1 and Th1 activity, greater fat production, and reduced fat breakdown. The liver shows elevated inflammation, lipogenesis, lipolysis, gluconeogenesis, and glycolysis. Muscle tissue shows reduced glucose uptake and lower GLUT4 expression. The brain displays blood-brain barrier dysfunction, increased neural inflammation, and circadian disruption. Brown adipose tissue is affected by altered batokine signaling, transcriptional reprogramming, and reduced thermogenesis. BACK.

Description 14: The vertical plot shows life expectancy in years, ranging from 0 to 50 in increments of 10. The horizontal plot shows the number of low-risk factors in five categories: none, one, two, three, and four or five. Each bar is divided into years lived without cancer, cardiovascular disease, or type 2 diabetes, and years lived with these conditions. In women, total life expectancy (LE) increases from about 30 years with no low-risk factors to about 43 years with four or five, with a greater portion of healthy years. In men, total LE rises from

roughly 31 to 39 years, also with more disease-free years as low-risk factors increase. BACK.

Description 15: The top illustration shows white blood cells, which occupy the largest segment. Other cell types, such as epithelial cells, red blood cells, fibroblasts, endothelial cells, stem cells, pericytes, neurons, glial cells, myocytes, gametes, and other striated myocytes, are represented by smaller segments. The labels for these cell types are placed around the circle, with lines connecting them to their respective segments. The bottom illustration highlights adipocytes, which dominate the chart as the largest segment. Other cell types, including epithelial cells, red blood cells, white blood cells, fibroblasts, neurons, glial cells, other striated myocytes, osteoid cells, gametes, smooth myocytes, pericytes, and stem cells, are displayed in smaller proportions. Similar to the top illustration, labels are placed around the circle. BACK.

Description 16: The scale bar marked “50 micrometers” appears in the bottom right corner. The myocyte is in cross-section with striated muscle fibers bundled together. Erythrocytes appear as smooth, biconcave red blood cells. Platelets are tiny, irregular fragments. Neutrophils are round multi-lobed nuclei in a sequence. Endothelial cells form a flat, continuous sheet representing a vessel lining. Alveolar cells are thin with irregular shapes and central nuclei. The neuron has a large central body, multiple dendrites, and a myelinated axon. Stem cells are round and minimally differentiated, arranged linearly. Adipocytes are large, round fat cells filled with a central lipid droplet. Fibroblasts are star-shaped with central nuclei. Hepatocytes form rows of polygonal cells, each with a central nucleus. BACK.

Description 17: The top panel is a scatter plot with T2D prevalence (percent) on the vertical plot and polygenic score percentile on the horizontal plot. Prevalence remains near zero at low percentiles and rises sharply after the nineteenth percentile, peaking at the hundredth. The bottom panel is a line plot showing the odds ratio for T2D on the vertical plot and the polygenic score percentile (gPRS) on the horizontal plot. The odds ratio is near 1.0 at low percentiles,

exceeds 2.0 by the fiftieth percentile, and rises above 5.0 beyond the 90th. Error bars show variability. Below, control counts stay near 12,400; T2D cases increase from 2,962 to 13,635. BACK.

Description 18: The vertical plot is labeled “Mortality Rate, percent,” ranging from 0 to 50 in increments of 5, and the horizontal plot is labeled “Person-Month Follow-Up,” ranging up to 150 months. Six lines are plotted: (1) normal weight with MetS shows the highest mortality, followed by (2) overweight with MetS and (3) obese with MetS. These three lines are higher throughout. In contrast, (4) normal weight without MetS, (5) overweight without MetS, and (6) obese without MetS show lower mortality, with the obese without MetS group having the lowest rates. Mortality differences widen over time, emphasizing higher risk with MetS regardless of weight status. BACK.

Description 19: At the top left is the structural model of the glucagon-like peptide-1 (GLP-1) receptor, displayed as a transmembrane helical protein arrangement. To its right is the structure of the gastric inhibitory polypeptide (GIP), illustrated as a single helical peptide chain. Below, the chemical structure of semaglutide features a long peptide chain with functional groups including hydroxyl groups, amide bonds, a phenyl ring, and a fatty acid side chain for improved pharmacokinetics. Adjacent is the structure of tirzepatide, which displays a more complex peptide sequence with multiple aromatic rings, amide bonds, and a fatty acid side chain. Tirzepatide is designed as a dual agonist for both GLP-1 and GIP receptors. BACK.

Description 20: The vertical plot shows death rates ranging from 10 to 60 deaths per 10,000 people. The horizontal plot spans years from 1958 to 2010. The heart disease line begins at 56 deaths per 10,000 people in 1958 and steadily declines to 18 in 2010, marking a 68 percent reduction. The stroke line starts at 19 deaths per 10,000 in 1958 and drops to 4 by 2010, a 79 percent reduction. The cancer line remains relatively stable, peaking around 1990, then declining to 17 deaths per 10,000 in 2010, a 10 percent reduction from 1958 and 20 percent

from the peak. Annotations beside each line summarize the changes, and small person icons visually depict the magnitude of the reductions. BACK.

Description 21: The vertical plot ranges from 250 to 500 in increments of 50, and the horizontal plot lists years from 2010 to 2022. The cardiovascular disease line begins at 456.7 in 2010, steadily declines to 416.0 in 2019, and then rises to 454.5 in 2022. The heart disease line starts at 347.3 in 2010, drops to 313.1 in 2019, and increases to 338.1 in 2022. Both lines follow a similar pattern, with decreasing mortality rates until 2019 followed by a reversal and increase through 2022. BACK.

Description 22: The vertical plot represents prevalence in percentages from 0 to 100. The horizontal plot lists age groups: 13 to 19, 20 to 29, 30 to 39, 40 to 49, and 50 years or older. For the 0.5-millimeter threshold, prevalence rises from 17 percent in ages 13 to 19 to 85 percent in those 50 or older. For the 0.3-millimeter threshold, prevalence is higher in each group, increasing from 21 percent in the youngest group to 91 percent in the oldest. The plot shows age-related increases in atherosclerosis, with higher detection at the lower thickness threshold. BACK.

Description 23: The vertical plot of the plot shows adjusted probability ranging from 0.00 to 0.20, and the horizontal plot shows the years from 0 to 8. Five lines represent different coronary artery conditions: “No SCA” has the lowest and flattest curve, while “Obstructive-extensive” shows the steepest rise and highest probability. Intermediate lines represent increasing risk from “Nonobstructive-nonextensive” to “Nonobstructive-extensive” and “Obstructive-nonextensive.” Below the plot, five labeled circular illustrations depict corresponding artery conditions. “No subclinical coronary artery disease” shows clear arteries. “Nonobstructive-nonextensive” displays one small blockage. “Nonobstructive-extensive” shows several small blockages. “Obstructive-nonextensive” features a single large blockage. “Obstructive-extensive” displays multiple large blockages. A central stylized heart visually connects the conditions to heart health. BACK.

Description 24: The vertical plot shows the percent reduction in major vascular events, ranging from minus 10 to 50 percent, while the horizontal plot shows the reduction in low-density lipoprotein (LDL) cholesterol in millimoles per liter, ranging from 0 to 2.0. Each square represents a clinical trial, with size indicating its weight and error bars reflecting confidence intervals. Trials such as JUPITER and 4S show large reductions in both LDL cholesterol and vascular events. HPS, ODYSSEY OUTCOMES, and FOURIER cluster around moderate reductions. Trials like GISSI and ALLHAT-LLT show smaller changes. A diagonal trend line illustrates a positive correlation between LDL cholesterol reduction and lower vascular event rates, with greater cholesterol reduction generally linked to greater risk reduction. BACK.

Description 25: The vertical plot shows the cumulative incidence rate in percent, ranging from 0 to 15. The horizontal plot shows time in years since enrollment, ranging from 0 to 4. Five lines represent different achieved low-density lipoprotein cholesterol (LDL-C) level groups: less than 20, 20 to less than 40, 40 to less than 55, 55 to less than 70, and 70 or greater. The line for LDL-C less than 20 shows the lowest cumulative incidence of cardiovascular death, myocardial infarction, or stroke, remaining under 5 percent by year 4. Incidence increases with each higher LDL-C group, with the LDL-C 70 or greater line reaching nearly 15 percent. Line shading darkens with higher LDL-C groups. A p-value of 0.0001 is marked. BACK.

Description 26: At the top, a comparison shows that the colchicine group (0.5 milligrams daily) had a 4.2 percent incidence of major adverse cardiovascular events (MACE), with 250 cases among 5,806 participants, while the placebo or no colchicine group had a 5.7 percent incidence, with 328 cases among 5,788 participants. This corresponds to a 25 percent relative risk reduction, with a relative risk of 0.75 and a 95 percent confidence interval of 0.61 to 0.92. The lower section details outcomes: myocardial infarction (relative risk (RR) 0.78, confidence interval (CI) 0.64 to 0.94), stroke (RR 0.54, CI 0.34 to 0.86), coronary revascularization (RR 0.77, CI 0.66 to 0.90), and cardiovascular death (RR 0.82, CI 0.55 to 1.23), which was not statistically significant. BACK.

Description 27: The vertical plot shows cardiac mortality percentage, ranging from 0 to 40 percent. The horizontal plot shows time in years, ranging from 0 to 10. Four lines represent different numbers of inflamed arteries: 0, 1, 2, and 3. The line for 0 arteries remains at 0 percent. The line for 1 artery rises gradually to about 13 percent. The line for 2 arteries increases more steeply, reaching 20.4 percent, and the line for 3 arteries climbs sharply to 29.8 percent by year 10. Reported hazard ratios with 95 percent confidence intervals are: 1 artery (13.0, 5.85 to 28.8), 2 arteries (20.4, 9.35 to 44.7), and 3 arteries (29.8, 13.9 to 63.9), all with p-values less than 0.001. BACK.

Description 28: The first section, “Identify High-Risk Individuals,” lists tools for risk detection, including clinical and lab factors, polygenic and microbiome risk scores, proteomic scores, heart organ scores, whole genome sequencing, hs-CRP, AI-analyzed retina photos and chest X-rays, epigenetic age, new biomarkers, and lifestyle and environmental factors. The second section, “Active Surveillance,” includes coronary computed tomoploty angioploty (CTCA), biomarkers, and timing interventions using multimodal artificial intelligence. A small plot shows increasing detection over time, with “Detection” on the vertical plot and “Time” on the horizontal plot. The third section, “Preventive Strategies,” recommends lifestyle changes, high-intensity statins, anti-inflammatory therapy, and gut microbiome modulation to lower coronary artery disease risk. BACK.

Description 29: The vertical plot shows subclonal frequencies, while the horizontal plot represents the evolutionary time from zygote to diagnosis, marked by a vertical dashed line. The timeline begins with a zygote, followed by the appearance of driver mutations, shown as stars. These lead to the formation of a cancer founder cell. From this point, branching shaded regions represent subclonal populations that expand and diverge over time. Each subclone is illustrated as a cell with additional mutations. The illustration ends at diagnosis, and a scale bar indicates approximately 500 mutations accumulated during this process. BACK.

Description 30: The vertical plot shows cancer mortality rates per 100,000 people, and the horizontal plot shows years from 2000 to 2020. Each plot represents a different age group. For ages 15 to 39, the line rises from about 3 to 6 deaths per 100,000, an 80 percent increase. For ages 40 to 64, the line shows a modest decline from roughly 46 to 40 deaths per 100,000, a 13 percent decrease. For ages 65 to 74, mortality drops significantly from around 85 to 40 deaths per 100,000, a 53 percent decrease. For ages 75 and over, the steepest drop is seen, from approximately 300 to 135 deaths per 100,000, a 55 percent reduction. BACK.

Description 31: For men, prostate cancer leads with 299,010 cases, followed by lung and bronchus (116,310) and colon and rectum (81,540). Other top types include bladder, skin melanoma, kidney, non-Hodgkin lymphoma, oral cavity, leukemia, and pancreas. For women, breast cancer ranks highest with 310,720 cases, followed by lung and bronchus (118,270) and colon and rectum (71,270). Other leading types include uterine corpus, skin melanoma, non-Hodgkin lymphoma, pancreas, thyroid, kidney, and leukemia. Lung and bronchus cancer is the second most common cancer for both men and women. BACK.

Description 32: The first section, “Identify High-Risk Individuals,” lists approaches including electronic health record analysis, lab result trends, serial imaging comparisons, polygenic risk scores, whole genome sequencing, lifestyle and environmental assessments, clonal hematopoiesis detection, high-sensitivity C-reactive protein measurement, proteomic scores, and new biomarkers. The second section, “Active Surveillance,” includes multi-cancer early detection tests, imaging, biomarker analysis, immunome profiling, and using multimodal artificial intelligence to guide the timing of interventions. A small plot shows detection improving over time. The third section, “Preventive Strategies,” lists interventions such as aggressive lifestyle changes, immune system enhancement via vaccines, and anti-inflammatory therapies. BACK.

Description 33: The first plot, “All-Cause Mortality,” shows higher mortality in the aspirin group with a hazard ratio of 1.14 (95 percent confidence interval:

1.01 to 1.29). The second plot, “Deaths from Cancer,” also shows a higher incidence of aspirin, with a hazard ratio of 1.13 (95 percent confidence interval: 1.10 to 1.56). The third plot, “Major Bleeding Events,” shows the greatest difference, with a hazard ratio of 1.18 (95 percent confidence interval: 1.18 to 1.62) and a p-value less than 0.001. In all plots, the cumulative incidence ranges from 0 to 10%, and participant numbers decline over time. The aspirin group shows consistently higher risk across all outcomes. BACK.

Description 34: The vertical plot shows the proportion of attributable cancer cases, ranging from 0 to 40 percent. The horizontal plot lists risk factors. ‘All risk factors’ account for 40 percent of cases. Cigarette smoking is the top individual contributor at 19.3 percent, followed by excess body weight (7.6 percent), alcohol (5.4 percent), UV radiation (4.6 percent), and physical inactivity (3.1 percent). Smaller contributions come from human papillomavirus (1.8 percent), low fruit slash vegetable intake (1.4 percent), processed meat (1.0 percent), low fiber (0.9 percent), and H. Pylori (0.7 percent). Other factors include red meat, hepatitis C virus, human immunodeficiency virus, low dietary calcium intake, exposure to secondhand tobacco smoke, Epstein-Barr virus, hepatitis B virus, and human herpesvirus 8. BACK.

Description 35: The vertical plot shows the proportion of the maximum value for each brain structure from 0 to 100 percent. The horizontal plot represents age in years on a logarithmic scale from conception to late adulthood. Gray matter volume rises rapidly before birth, peaks in late infancy, and then declines steadily. White matter volume increases from birth, peaks in young adulthood, and decreases slightly later. Ventricular volume stays low until late adulthood, then rises sharply. Cortical thickness peaks in late infancy and gradually declines throughout life. Key life stages mid-late fetal, late infancy, late childhood, young adulthood, and late adulthood are marked on the age axis. BACK.

Description 36: The vertical plot shows levels of activity of biological markers. The horizontal plot represents time, progressing from no disease to advanced symptomatic Alzheimer’s disease, with stages marked by Clinical Dementia

Rating scores from 0 to 3. Four curves are depicted: synaptic and neuronal function starts high, then gradually declines and sharply drops after cognitive symptoms begin; amyloid-beta deposition rises early, peaks before symptoms, then plateaus; microglia and astrocyte activation rises during preclinical Alzheimer's disease, peaks near symptom onset, and remains high; tau pathology begins rising later, increases rapidly after symptom onset, and continues to rise. The transition from preclinical to symptomatic Alzheimer's disease is indicated by cognitive impairment onset. BACK.

Description 37: The vertical plot shows the hazard ratio for dementia from 0 to 10 in increments of 1. The horizontal plot represents sleep duration from 4 to 9 hours in increments of 1. A solid curve forms a U shape, with a hazard ratio of around 3 at 4 hours, dropping to 1 near 7 hours, then rising to about 2 at 9 hours. Dashed lines indicate the 95 percent confidence interval, which widens notably at 4 and 9 hours, reflecting greater uncertainty at these extremes. The lowest dementia risk corresponds to approximately 7 hours of sleep, with a higher risk linked to shorter or longer durations. BACK.

Description 38: Early life risk factor elimination contributes 5 percent. Midlife factors offer the greatest impact: hearing loss and high low-density lipoprotein cholesterol each contribute 7 percent, followed by depression and traumatic brain injury at 3 percent each. Additional midlife contributors are poor sleep health, physical inactivity, diabetes, smoking, hypertension, and ultraprocessed foods, each adds 1 to 2 percent, along with obesity and excessive alcohol at 1 percent each. Late-life risk factors include social isolation (5 percent), air pollution (3 percent), visual loss (2 percent), and severe COVID-19 (2 percent). Combined, these modifiable factors exceed a 50 percent potential reduction. BACK.

Description 39: The first section, "Identify High-Risk Individuals," lists factors and methods for identifying those at risk. These include clinical factors, lifestyle factors, APOE genotype, polygenic risk score, retina exam, p-tau-217 (a biomarker), inflammation blood markers, brain organ clock, and epigenetic age.

The second section, “Active Surveillance,” highlights methods for monitoring individuals. These include retina imaging, biomarkers, brain MRI, brain PET scans, brain organ clock, and assessments of vision and hearing loss. A small plot in this section shows detection increasing over time. The third section, “Preventive Strategies,” outlines approaches to reduce the risk of Alzheimer’s. These include aggressive lifestyle modifications, immune and inflammation modulators, anti-amyloid agents, and gut microbiome modulation. BACK.

Description 40: The timeline spans from age 0 to 70 years and beyond, divided into early life, adult life, midlife, and late life. At the top, the “Risk factors” include “Causative and risk genes” and “alpha-synuclein aggregation.” Below this, a horizontal bar labeled “Neurotoxicants” spans early life to midlife, transitioning into “Neurodegeneration” in late life. At the far right, “Parkinson’s disease (symptomatic)” is marked in late life. A lower section highlights “Protective exposures (e.g., physical activity)” as a continuous bar spanning all life stages. This bar is divided into three phases: “Primary prevention” during early life, “Secondary prevention” during adult and midlife, and “Symptomatic prevention” in late life. Below this, “Neuroprotection” is emphasized. At the bottom, a box labeled “Protective factors.” BACK.

Description 41: At the top, a deoxyribonucleic acid strand is shown with a specific gene region targeted by the clustered regularly interspaced short palindromic repeats-associated protein 9 complex. The associated protein 9 enzyme, depicted as a scissor-like structure, binds to the deoxyribonucleic acid and induces a double-stranded break at the targeted site, labeled “Double-stranded break.” Below, the illustration shows two possible outcomes of the repair process via end joining. On the left, the deoxyribonucleic acid strand is repaired with additional nucleotides inserted, labeled as “Random insertions,” represented by small T-shaped symbols. On the right, the strand is repaired with missing nucleotides, labeled as “Random deletions,” also represented by T-shaped symbols. BACK.

Description 42: In base editing, a deaminase enzyme guided by nickase clustered regularly interspaced short palindromic repeats-associated protein 9 converts adenine to inosine, read as guanine, changing an adenine-thymine pair to guanine-cytosine. In prime editing, prime editing guide ribonucleic acid inserts a new sequence after nicking the strand, followed by repair of the complementary strand. Both methods avoid double-stranded breaks and enable precise sequence changes. Labels and arrows indicate each step. BACK.

Description 43: The lipid nanoparticle is made of ionizable lipids, helper lipids, cholesterol, and polyethylene glycol-lipids. Inside, it carries messenger ribonucleic acid encoding a gene-editing protein and chemically modified guide ribonucleic acid. The messenger ribonucleic acid appears as a wavy line, while the guide ribonucleic acid is structured with marked modifications: N1-methylpseudouridine, 2-prime-O-methylation, and phosphorothioate linkages. A zoomed-in section highlights the guide ribonucleic acid structure and labels each modification. The labeled lines point to their positions in the nanoparticle. BACK.

Description 44: In the “ON” state (top), deactivated clustered regularly interspaced short palindromic repeats-associated protein 9, guided by a ribonucleic acid sequence, binds to a target deoxyribonucleic acid region. An attached effector protein promotes gene activation by adding histone markers, displayed as hexagons, and modifying the deoxyribonucleic acid. In the “OFF” state (bottom), deactivated clustered regularly interspaced short palindromic repeats-associated protein 9 and its effector protein repress gene expression by removing histone markers and modifying deoxyribonucleic acid to block transcription. Arrows show activation or repression direction. Labels identify deoxyribonucleic acid, histone, deactivated clustered regularly interspaced short palindromic repeats-associated protein 9, effector protein, deoxyribonucleic acid modification, and histone marker. BACK.

Description 45: The central human figure with overlaid organ systems and surrounding labeled compartments represents immune cell distribution, totaling

approximately 1.5 to 2.3 trillion cells with a 95 percent confidence interval. Compartments include blood (40 billion cells), liver (50 billion), gastrointestinal tract (50 billion), bone marrow (700 billion), lungs (70 billion), lymphatic system (700 billion), skin (80 billion), and others (90 billion). Each section has a grid of squares, each representing 1 billion cells. The squares are color-coded to indicate immune cell types: T cells, B cells, plasma cells, natural killer cells, mast cells, neutrophils, eosinophils, basophils, macrophages, monocytes, and dendritic cells. Bone marrow and the lymphatic system contain the most cells. A legend links each color to its cell type. BACK.

Description 46: The top features illustrations of 12 immune cell types, T cells, B cells, plasma cells, natural killer cells, mast cells, neutrophils, eosinophils, basophils, macrophages, monocytes, dendritic cells and two components, complement and antibodies. Each has a distinct shape and texture. The bottom section presents a table titled "The total population of immune cell types in the body," listing total numbers, 95 percent confidence intervals, total mass in grams, and mass confidence intervals. T cells and macrophages are the most abundant. The total immune cell population is 1.8 trillion (1.5 to 2.3 trillion range), with a mass of 1,200 grams (800 to 1,900 range). BACK.

Description 47: At the top left, a nanoparticle is depicted as a central dark sphere surrounded by branching structures labeled "Antigen." An arrow points from the nanoparticle to a T cell, represented as a large circular structure with smaller protrusions labeled "Major histocompatibility complex." Another arrow extends from the T cell to a group of three smaller circular cells labeled "Regulatory T cell," indicating the differentiation process. BACK.

Description 48: The timeline titled "From lab to jab," shows the time between disease identification and vaccine approval in the United States from 1880 to 2020. Each disease is marked with a filled circle for the year the infection was linked and an open circle for vaccine licensing. Malaria, acquired immunodeficiency syndrome, and Zika fever have no licensed vaccines as of 2020. Tuberculosis took 39 years, typhoid fever 105 years, and dengue fever 112

years. COVID-19 progressed the fastest, with its virus identified in 2019 and a vaccine licensed by 2020. The timeline shows COVID-19 vaccine development was significantly faster than for previous diseases. BACK.

Description 49: The viruses include severe acute respiratory syndrome coronavirus 2, respiratory syncytial virus, influenza, Ebola, Zika, rabies, and human immunodeficiency virus. Components labeled include spike proteins, membranes, matrix proteins, nucleocapsids, enzymes like polymerase and reverse transcriptase, and viral glycoproteins such as gp120 and gp41. The malaria parasite, Plasmodium gametocyte, is oval-shaped with a labeled vacuole, erythrocyte membrane, and nucleus. Each organism displays unique shapes, spherical, filamentous, or bullet-shaped, and distinct structural proteins essential for infection and replication. BACK.

Description 50: At the top is the capsid, a geometric, icosahedral structure represented as coiled strands inside the capsid. Below the capsid is the tail, which consists of a long, cylindrical tail tube. The tail tube is segmented and connects the capsid to the baseplate at the bottom. The baseplate is a flat, circular structure that serves as the attachment point for the tail fibers. Extending outward from the baseplate are several tail fibers, which are thin, leg-like structures. A central tail fiber or spike protrudes downward from the baseplate. BACK.

Description 51: The illustration starts with a test tube labeled “Metagenomic sample containing a mix of microbes,” which contains various microbial shapes. An arrow labeled “Extraction and processing” points to a second test tube labeled “Library containing a mix of short deoxyribonucleic acid (DNA) fragments,” which contains DNA strand illustrations. Another arrow labeled “Sequencing” leads to a box labeled “Unclassified sequenced DNA reads,” containing sequences such as “ATACATGACA” and “CCTATATATTAT.” From this box, an arrow labeled “Classification” points to a second box labeled “Classification to their species of origin, and an abundance profile.” This box

contains DNA sequences and a bar chart with varying heights, representing the abundance of different microbial species. BACK.

Description 52: The top plot shows the odds of reporting good or very good health; the bottom shows the odds of reporting high well-being. Both use the same horizontal categories: 0, 1 to 59, 60 to 119, 120 to 179, 180 to 239, 240 to 299, and 300 or more minutes. The vertical plot in both plots ranges from 0.60 to 2.00. Odds ratios peak at 120 to 179 minutes above 1.40 for health and above 1.30 for well-being, then slightly decline. Zero minutes serve as the baseline (1.00). Error bars show variability. Both plots suggest that 120 to 179 minutes offers the greatest benefits, with reduced gains at longer durations. BACK.

Description 53: The top diagram shows eight factors contributing to molecular clock alterations around a central brain: inadequate sleep (bed icon), circadian misalignment (person under artificial light), brain activity changes (brain icon), lifespan changes (figures of different ages), genetic vulnerability (deoxyribonucleic acid strand), poor diet (pizza and drink), lack of physical activity (gaming console), and inappropriate light exposure (lightbulb). The bottom diagram presents eight interventions to address these changes, also circling a brain: cognitive behavioral therapy for insomnia (person in therapy), minimizing circadian misalignment (person in sunlight), neurostimulation (brain icon), timed melatonin (moon and stars), personalized interventions (group of people), timed medication (pill bottle), healthy diet (fruits and vegetables), and physical activity (bicycle). BACK.

Description 54: The interventions are listed on the left with participant numbers in boxes: walking or jogging (1,210), cognitive behavioral therapy (712), yoga (1,047), exercise with selective serotonin reuptake inhibitors (268), aerobic exercise with therapy (404), strength training (643), mixed aerobic exercises (1,286), tai chi or qigong (343), aerobic plus strength training (1,036), and selective serotonin reuptake inhibitors alone (432). The horizontal plot ranges from minus 1.5 to 1.5, with zero as neutral. Most interventions show negative mean differences, suggesting positive effects, especially walking or

jogging, mixed aerobic exercises, and aerobic plus strength training. Selective serotonin reuptake inhibitors alone show minimal effect. BACK.

Description 55: The line plot with time in minutes on the vertical plot (0 to 500) and age on the horizontal plot (starting at 15, increasing by 10-year intervals). The time spent alone rises steadily with age, sharply increasing after 50 and peaking above 400 minutes by age 80. Time with a partner grows in early adulthood, remains around 200 minutes from ages 30 to 60, and then declines. Time with family and friends declines steadily after age 15, dropping below 50 minutes after age 40. Time with children peaks in the 30s, nearly 100 minutes, and falls to near zero by age 60. Time with coworkers peaks in the 20s and also approaches zero by age 60. BACK.

Description 56: At the center, silhouettes depict human life stages from fetus to elderly adult. The outer ring has labeled segments for each hallmark. Primary hallmarks include genomic instability (deoxyribonucleic acid icon), telomere attrition, epigenetic alterations (coiled structure), and loss of proteostasis. Antagonistic hallmarks include deregulated nutrient sensing (fork and spoon icon), mitochondrial dysfunction (mitochondrion), and cellular senescence. Integrative hallmarks include stem cell exhaustion (stem cell icon), altered intercellular communication (interconnected circles), chronic inflammation, and dysbiosis (bacterium). BACK.

Description 57: Eight animals arranged by increasing lifespan, displayed in two rows with illustrations and labeled lifespans. Top row: African killifish (0.75 years), mouse (4 years), naked mole rat (31 years), and Brandt's bat (40 years). Bottom row: elephant (65 years), human (122 years), bowhead whale (200 years), and Greenland shark (392 years). Each is drawn simply and positioned left to right from shortest to longest lifespan. BACK.

Description 58: The vertical plot represents the quantity of proteins associated with aging, while the horizontal plot represents time. The first peak is relatively small, followed by a decline. The second peak is moderate in size, occurring after

a period of stability. The third peak is the largest and most prominent, followed by a sharp decline. BACK.

Description 59: The bar plot shows increased mortality risk over fifteen years by organ age gap, from negative twenty to positive eighty percent. Organs include the heart, arteries, brain, pancreas, liver, muscle, intestine, lung, and kidney. The heart and artery show the highest risk; the kidney the lowest. The survival curve shows probability from zero to one over fifteen years for groups: Brain or Immune, Normal age, Multiorgan two to four or more, five to seven or more, and eight or more. Higher multiorgan aging links to faster survival decline. Shading indicates confidence intervals. BACK.

Description 60: The first plot, labeled “White matter,” has a curved line that rises and then falls. Below it is an icon of a head with a brain outline. The second plot, “Cognitive health,” has a downward-sloping dashed line with a head and gear icon beneath. The third plot, “Episodic memory,” has a gradually declining line with an icon of a head and thought bubbles. BACK.

Description 61: The vertical plot shows lifetime gain from 0 to 6 years in 1-year increments. The horizontal plot shows age from 40 to 100 years in 10-year increments. Each plot includes six lines for lifestyle point ranges: 0 to 2 (reference), 3, 4, 5, 6, and 7 to 8. In men, the 7 to 8-point line peaks at 5.5 years at age 40. In women, it peaks at 5 years. Lifetime gain decreases with age. A statistically significant relationship is noted with P less than 0.001. BACK.

Description 62: The vertical plot represents years of life gained, ranging from 0 to 5 in 1-year increments. The horizontal plot represents metabolic equivalent task hours per week, ranging from 0 to 30 in 5-unit increments. The curve rises steeply up to 10 metabolic equivalent task hours per week, reaching about 3 years gained, then flattens, with gains leveling off around 4.5 to 5 years between 20 and 30 metabolic equivalent task hours. Error bars are present at each data point. BACK.

Description 63: The vertical plot represents deoxyribonucleic acid methylation GrimAge in years, ranging from 20 to 100 in 10-year increments. The horizontal plot represents chronological age in years, also ranging from 20 to 100 in 10-year increments. Two lines are shown: a solid line for the control group and a dashed line for people who exercise. Both lines trend upward, but the dashed line remains consistently below the solid line, indicating lower deoxyribonucleic acid methylation GrimAge for the exercise group across all ages. BACK.

Description 64: The first plot shows survival by age for 1900, 1980, and an ideal case. The vertical plot shows percent surviving from 0 to 100 percent; the horizontal plot shows age from 0 to 100 years. The 1900 line drops steeply before age 50, the 1980 declines after 70, and the ideal line stays at 100 percent until 90. Shaded areas are labeled “Trauma” and “Ideal.” The second plot shows health span versus lifespan. Both axes range from 0 to 100 percent. The “No intervention” line declines early, “Medical intervention 2.0” declines slowly, and “Medical intervention 3.0” remains near 100 percent before dropping. BACK.

Description 65: The vertical plot represents transformative impact, ranging from low to high. The horizontal plot represents time. The plot features two exponential growth curves: one for artificial intelligence and one for digital biology. Both start with low impact and rise rapidly over time. The artificial intelligence curve is slightly ahead, indicating it reaches a higher transformative impact earlier than digital biology. BACK.

Description 66: The vertical plot of the top plot shows a naive cluster of differentiation 8 positive T cell frequency, ranging from 0 to 60. The horizontal plot shows age from 60 to 100 years. Data points are shaded squares, and a regression line with a shaded confidence interval slopes downward, indicating a decline in cell frequency with age. The vertical plot of the bottom plot shows the immune health metric, ranging from 0.1 to 0.5. The horizontal plot shows age from 0 to 60 years. Data points are black circles with a regression line showing a negative trend. A correlation coefficient of negative 0.56 and a p-value of 0.00057 are displayed. BACK.

Description 67: The vertical plot of the scatter plot shows fold-over-background values from 0 to 20 in 5-unit increments. The horizontal plot shows the position from 0 to 120,000 in 20,000-unit increments. Black data points are scattered, with denser clusters below 40,000 and near 120,000. A density plot below the axis shows point distribution. The adjacent table, titled “Virus Exposure Aggregate Reactivity Score,” lists ten viruses with scores for the Topol and Control groups. Examples include: Rhinovirus A (Topol 16, Control 20), Epstein-Barr virus (Topol 10, Control 22), and Herpes Simplex virus (Topol 10, Control 1.2). BACK.

Description 68: The vertical plot of the scatter plot shows fold-over-background values starting at 0, increasing in consistent increments. The horizontal plot shows the position from 0 to 30,000 in 5,000-unit increments. The dots represent data points, with most clustered between positions 20,000 and 30,000, indicating higher responses in that range. Below, a table compares fold-over-background values between the Topol and Control groups. Zinc finger and BTB domain-containing protein 5 shows the highest Topol value at 50.3 versus 1 in Control. Other proteins, like fibronectin type 3 domain-containing protein 4 and ubiquitin-conjugating enzyme E2, also show elevated Topol values. BACK.